Title: HETEROLOGOUS PLASMA COCKTAIL FOR HIV TREATMENT

Abstract: The present invention is to a heterologous plasma cocktail and a method to treat and/or prevent HIV infection with the cocktail.
Heterologous Plasma Cocktail for HIV Treatment

This application claims priority to US 60/418136, which was filed on October 11, 2002.

Field of the Invention

The present invention provides compositions and methods for treating and preventing viral infection, and in particular for treating and preventing infection with the human immunodeficiency virus (HIV).

Background of the Invention

The first clinical evidence of AIDS (Acquired Immune Deficiency Syndrome) was documented by the Center for Disease Control and Prevention (CDC) in 1981, with reports of Kaposi's Sarcoma (KS) and Pneumocystis Carinii Pneumonia (PCP) in previously healthy homosexual men. At the time, both KS and PCP were known only as opportunistic diseases found in immunodepressed patients. From the earliest reports of the new disease syndrome, scientists around the world focused their efforts on finding the cause. Within only a few years, scientists at the National Cancer Institute and the Pasteur Institute separately identified a virus referred to as human immunodeficiency virus (HIV) as the etiologic agent responsible for AIDS. Gallo, R. et al., Science, 224:500-503 (1984); Barre-Sinoussi, F. et al., Science, 220:868-870 (1983).

HIV is a retrovirus, which as a class of viruses are small enveloped viruses that contain a diploid, single-stranded RNA genome, and replicate through a DNA intermediate produced by a virally-encoded enzyme known as reverse transcriptase (RT). Varmus, H., Science 240:1427-1439 (1988). There are two types of HIV, HIV-1 and HIV-2, which differ in the ability to cause disease and in geographical distribution. HIV-1 is by far the more dominant of the two.

Within the retrovirus family, HIV is classified as a lentivirus. Among retroviruses, lentiviruses are known for the complexity of their viral genomes. While most retroviruses contain only three genes (i.e., env, gag and pol), HIV contains these essential genes as well as complex regulatory genes (i.e., tat, rev, nef) and auxiliary genes (i.e., vif, vpr and vpu).

Lentiviruses are known in other species, including the simian immunodeficiency virus (SIV), caprine arthritis-encephalitis virus (CAEV), and feline immunodeficiency virus (FIV).
Cells of the immune system, particularly CD4+ T cells (i.e., Th1 type cells which are primarily involved cytotoxic T cell immunity) are the primary targets of HIV infection. CD40 serves as the cellular receptor for HIV. Primary HIV infection is associated with a burst of HIV viremia and often a concomitant abrupt decline of CD4+ T cells in the peripheral blood. The typical course of infection with HIV, like most lentiviruses, involves a long incubation period between initial infection and the manifestation of clinical disease. The median period of time between infection with HIV and the onset of clinically apparent disease is approximately 10 years in western countries. As the infection runs its course, the T-helper cells become depleted and progressive impairment of the immune system occurs, resulting in the development of opportunistic diseases and ultimately death.

In the two decades since its discovery, HIV has wreaked global devastation. The syndrome now known as AIDS includes more than 25 AIDS associated conditions or diseases. More than 60 million people have been infected with HIV, and currently more than 40 million people are estimated to be living with HIV/AIDS. An estimated 5 million people became infected with HIV in 2002, and more than 95% of these new infections were in developing countries. UNAIDS. AIDS Epidemic Update, December, 2002. The disease is the now the fourth leading cause of death worldwide. Deaths among those already infected will continue to increase for some years even if prevention programs reduce the number of new infections. With the HIV-positive population still expanding, the annual number of AIDS deaths can be expected to increase for many years. AIDS is disproportionately a disease of the young, with more than half of HIV POSITIVE individuals infected before the age of 25 and succumbing to disease and ultimately death before the age of 35. As a result, more than 14 million children have been orphaned by AIDS.

Identification of HIV as the etiologic agent of AIDS led to the development of antivirals and other therapies. As early as 1986, preliminary reports on the use of 3'-azido-3'-deoxy-thymidine were presented at the Second International AIDS Conference. That drug (AZT, Zidovudine, Retrovir) became the first therapeutic approved by the Food and Drug Administration (FDA) for use in treatment of HIV.

Other therapeutics quickly followed. Over the next eight years, sixteen HIV antiretroviral agents were approved by the FDA, and several others are available through expanded access programs. Most of these approved HIV therapeutics are reverse transcriptase inhibitors which can be either nucleoside or non-nucleoside RT inhibitors ("NRTIs or NNRTIs").
NRTIs, the class that includes the first drug approved by the FDA, are analogs of normal nucleotides that act as building blocks by reverse transcriptase in the process of assembling DNA from viral RNA. These aberrant nucleoside triphosphates are incorporated into the transcribed DNA chain, preventing elongation, stopping viral replication, or simply act as enzyme inhibitors. NRTIs include AZT (Zidovudine, Retrovir, GlaxoSmithKline), Epivir (3TC, β-L-2',3'-dideoxy-3'-thiacytidine, GlaxoSmithKline); Emtriva (FTC, β-L-2',3'-dideoxy-3'-thia-5-fluorocytidine, Gilead Sciences, Inc.); Didanosine (ddI, 2',3'-dideoxyinosine, Bristol Myers Squibb); Abacavir (Ziagen, GlaxoSmithKline); Stavudine (D4T, 2,3-dideoxy-β-D-glycerol-pent-2-en-o-furanosyl thymine, Bristol Myers Squibb), and Amdoxovir (DAPD, 2',3'-dideoxy-3'-oxa-2,6-diaminopurine, Gilead Sciences, Inc).

NNRTIs include Sustiva (efavirenz; Bristol Myers Squibb) and nevirapine (Viramune, BI-587, Boeringer Ingelheim). The NNRTIs can produce moderate levels of intolerance related to rash and central nervous system (CNS) effects, as well as infrequent but serious toxicity, (including severe cutaneous reactions and liver toxicity) and a low genetic barrier to resistance. NNRTIs are nevertheless prescribed because they can be tolerated for long periods if initial serious reactions do not occur. Development of NNRTIs continues as a source of therapeutic alternatives to HIV positive individuals who have developed resistance to those NNRTIs already on the market.

A second class of drugs targets the viral protease, an enzyme responsible for processing HIV-fusion polypeptide precursors. In HIV and several other retroviruses, the proteolytic maturation of the gag and gag/pol fusion polypeptides (a process indispensable for generation of infective viral particles) has been shown to be mediated by a protease that is, itself, encoded by the pol region of the viral genome. Y. Yoshinaka, et al., Proc. Natl. Acad. Sci. USA, 82:1618-1622 (1985); Y. Yoshinaka, et al., J. Virol., 55:870-873 (1985); Y. Yoshinaka, et al., J. Virol., 57:826-832 (1986); and K. von der Helm, Proc. Natl. Acad. Sci., USA, 74:911-915 (1977). Inhibition of the protease has been shown to inhibit the processing of the HIV p55 in mammalian cell and HIV replication in T lymphocytes. T. J. McQuade, et al., Science, 247:454 (1990). FDA approved protease inhibitors include Saquinavir (Invirase® Fortovase®, Roche); Ritonavir (Norvir®, Abbott); Indinavir (Crixivan®, Merck); Nelfinavir (Viracept®, Agouron); Amprenavir (Agenerase®, GlaxoSmithKline); Lopinavir (Kaletra®, Abbott); Atazanavir® (BMS 232632, Bristol-Myers Squibb). Other protease inhibitors in human trials include: GW433908 (GlaxoSmithKline), Tipranavir® (Boehringer Ingelheim); and TMC114 (Tibotec Virco).
The most notable side effects of protease inhibitors include nausea, diarrhea, headache, kidney stones and serious drug interactions. Protease inhibitors may also cause unusual fat deposits as well as high levels of fats and cholesterol in the bloodstream. Researchers have new and increasing concerns regarding protease inhibitors and their cross resistance. Cross-resistance occurs when changes which cause resistance to one drug also cause resistance to another.

The emergence of resistant HIV strains has led to the use of combination therapy, the use of two or more drugs as the same time in a drug “cocktail,” which has been shown to be effective at least initially to combat the high risk of resistance to any individual drug. Combination therapy is now the standard of care for people with HIV. It is sometimes called HAART (Highly Active Anti-Retroviral Therapy). Typically, combination therapy will include three selections, including some combination of NRTIs alone or in combination with a PI. The choice of which drugs to combine takes into account synergistic effects of the drug combinations, as well as other sorts of drug-drug interactions that might render a combination less effective or even dangerous. One of the issues that must be considered when developing a combination therapy is the likelihood of patient compliance with the prescribed regimen. The use of several drugs, each having certain restrictions regarding how often and when it must be taken (e.g., before or after meals, or with certain types of food), frequently results in a complicated medication schedule and consumption of large numbers of pills to be taken. Furthermore, each of the drugs is associated with a variety of side effects, which are generally related to the dosage level.

Considerable effort has been directed to develop drugs targeting integrase, the viral protein responsible for integration. Integrase is an attractive target for antivirals because, unlike protease and reverse transcriptase, there are no known counterparts in the host cell. Goldgur, Y, et al., Biochemistry, 96(23)13040-13043 (1999). Unfortunately, it has also proven to be a difficult target due to the complexity of the integration event. As a result, after more than a decade of work, there are no approved integrase inhibitors. There are, however, two drugs in early clinical trials including S-1360 (Shionogi Pharmaceuticals and GlaxoSmithKline) and L-870810 (Merck). Cross-resistance has been observed in initial experiments.
Fusion or entry inhibitors provide another novel mechanism of action. Unlike NNRTIs, NRTIs and PS, which are only active against HIV after it has entered a cell, fusion inhibitors prevent the virus from entering the cell. HIV entry can be broken down into three basic steps: (1) HIV binding via the gp120 envelope protein to the CD4 molecule on the Th1 cell surface; (2) a change in envelope protein conformation that leads to gp120 binding to a second receptor (either CCR5 or CXCR4); and 3) gp41-mediated fusion of the viral envelope with the cell membrane, completing viral entry. At least one inhibitor targeting each step in this pathway is currently in clinical development. Only one entry inhibitor, Fuzeon® (Trimeris), has been approved by the FDA for treatment of HIV. Fuzeon is a peptide therapeutic which works by binding to gp41. T1249 (Trimeris), another fusion inhibitor that works by binding to gp41, is also in human trials. Fusion inhibitors that bind to T-cell proteins are also in development, including PRO-542 (Progenics Pharmaceuticals) and BMS 806 (Bristol Myers Squibb). AMD070 (AnorMed) targets the CXCR4 chemokine receptor and is currently in Phase I. Development of another AnorMed fusion inhibitor, AMD3100, was halted in May of 2001 as a result of poor clinical results. SCH C (Schering Plough) is a CCR5 antagonist that blocks the interaction between the V3 loop of gp120 and the CCR5 receptor. The FDA has allowed further clinical development of this agent despite adverse cardiac events in early clinical trials.

Antisense drugs, another new class of therapeutics, block expression of viral genes using antisense oligonucleotides. Viruses are particularly suitable targets for antisense therapy because they carry genetic information distinct from the host cells. At present, two antisense drugs are in early clinical trials, including HGT843 (Enzo Biochem) and GEM-92 (Hybridon). Antisense technology, however, has produced only one marketable drug in the twenty years since it was first developed. Several HIV antisense programs have been abandoned as a result of drug delivery issues and dosage concerns. Whereas each of the aforementioned therapies for HIV launch an offensive attack against the virus, attention has also been given to defensive strategies for enhancing the immune system of HIV infected patients. It is known that patients mount a characteristic immune response with weeks to months of infection with HIV, including production of HIV-specific antibodies and expansion of HIV-1 specific CD4 and CD8 T cells. Moog, C et al., J Virol, 71(5):3734-41 (1997); Robert-Guroff, et al., Nature, 316(6023):72-4 (1985). Yet, it has been observed that this immune response is insufficient to control disease progression, perhaps due to the emergence of mutant virus in response to selective pressure from neutralizing antibodies.
Strategies for enhancing the immune response are varied. One strategy involves cytokine manipulation. Cytokines are the chemical messengers of the immune system, and include small proteins and biological factors such as interleukins, chemokines, lymphokines, interferons and other signaling molecules such as tumor necrosis factor. While the role of cytokines in disease progress is not clearly understood, cytokine profiles are clearly disturbed. Levels of certain cytokines (e.g., IL-1, IL-6, TNP-alpha, intefrons-alpha and gamma) increase, while others (e.g., IL-2) decrease. The best-known cytokine therapeutic is interleukin-2 (IL-2, Aldesleukin, Proleukin, Chiron Corporation). IL-2 is often used in combination with antiretroviral drugs or and during therapeutic “breaks” from antiretroviral therapy. Multikine (Cel-Sci Corporation) is a mixture of several different cytokines. HE2000 (Hollis-Eden Pharmaceuticals) is in Phase I/II trials. Reticulose (Advanced Viral Research Corporation) is a nucleic acid that stimulates the cell-killing arm of the immune system.

A second strategy for enhancing the immune system involves passive immunization using blood products derived from other animals or humans infected with HIV. Passive immunization has a long history in the treatment of disease beginning with the use of serum therapy in the late 1800’s. Behring and Kitasato were the first to use passive immunization in the treatment of diphtheria in 1890. At that time, serum was not known to contain antibodies; rather it was only observed that serum produced a beneficial therapeutic effect. In the 1920’s and 30’s, sera produced in animals (e.g., horse, rabbit, sheep) was used to treat patients with measles and scarlet fever. Serum therapy declined in popularity with the discovery of antibodies, and the development of strategies such as pooling and monoclonal antibody technology. Passive immunization against HIV has involved treating patients with heterologous neutralizing antibodies (HNabs). The term “heterologous” refers to the source of neutralizing antibodies as “other,” and can refer to other humans or animals. In general, HNabs are prepared by purification from the serum of HIV positive individuals, or by exposing a particular animal to HIV, permitting Nabs to develop, and then isolating those Nabs from the animal serum.

Blood is a liquid tissue containing cells, proteins, salts and various amounts of organic substances. The term plasma refers to that portion of the blood that remains after the cellular elements (i.e., red blood cells, white blood cells) have been removed, typically by centrifugation. Serum, in contrast, refers to the portion of the blood that remains after both the cellular elements and the clotting proteins are removed. Clotting will occur naturally when blood is added to a test tube, but can also be stimulated by clotting activators such as
calium. Serum contains water (98%), protein (6-8%), salts (0.8%), lipids (0.6%) and glucose (0.1%). Other molecules, including metabolites, hormones and enzymes are also present.

Serum should be therefore be distinguished from plasma, although the two terms are sometimes used interchangeably incorrectly. Those skilled in the art of blood collection are familiar with the differences between the two, which determine how blood samples are collected. To prevent conversion to serum, plasma is typically collected in tubes coated with an anticoagulant, or anticoagulant is added to the blood shortly after collection. Common additives used to prevent coagulation include heparin, EDTA, citrate or oxalate. Serum is collected in uncoated tubes and permitted to clot, or in tubes with clotting activators to speed the process.

Serum contains a complex mixture of proteins that contains various proteins ranging in concentration over at least 9 orders of magnitude. Adkins, JN et al., Mol Cell Proteomics 1(12):947-55 (2002). The most abundant serum proteins are albumins, which account for between 60-80% of total serum protein. Albumin is a small protein produced by the liver and responsible for transport of small molecules, such as calcium, around the body. Albumin also helps keep blood fluids from leaking out into tissue. Globulins are a second form of protein found in large quantities in serum. Larger than albumin, the globulins can be classified according to three major types: alpha, beta and gamma. Alpha and beta globulins mainly carry various lipids, lipid-soluble hormones and vitamins, and other lipid-like substances in the plasma. The alpha-1 fraction includes alpha-1 anti-trypsin and thyroxine binding globulin. The alpha-2 fraction contains haptoglobin, ceruloplasmin, HDL, and alpha-2 macroglobulin. The beta fraction includes transferrin. The gamma globulins consist primarily of the immunoglobulins (i.e., IgA, IgM, IgG). Protein levels may vary somewhat based on, for example, disease or nutritional state.

Numerous extraction and purification strategies have been developed to isolate the serum proteins with various degrees of specificity. These strategies exploit the differences among proteins with respect to such characteristics as size, charge, and binding affinity, among others. Representative technologies include chromatography (i.e., gel filtration, affinity and ion exchange), precipitation and gel electrophoresis. Robert K. Scopes, Protein Purification: Principles and Practice, 3rd edition, Springer Verlag (1994). These techniques can be used alone or in combination. For example, ionic precipitation (i.e., using ammonium sulfate) is most often used early in a purification to permit some subset of proteins to be fractionated from the whole based on common solubility parameters, and is often followed by
chromatography. Ionic precipitation can be used to obtain a crude extract of proteins that can then be further purified.

HNabs produced from the sera of HIV positive individuals have been used to treat HIV. Karpas, A et al., Proc Natl Acad Sci, 85:9234-7 (1988); Levy, J et al. Blood, 84(7):2130-5 (1994); Vittecoq, D et al., Proc Natl Acad Sci, 92(4):1195-9 (1995). US 4,863,730 (Karpas) teaches a method for treating from HIV positive individuals in which plasma is obtained from HIV positive patients and then processed to provide a preparation having a high titer of HNabs. Karpas distinguishes the unprocessed plasma (plasma as it is derived by separation from an individual’s blood, for example by centrifugation) from the “processed plasma” composition of the invention (which is processed to remove substantially all non-fluidic components other than certain antibodies). While some clinical reports suggest therapeutic benefit (i.e., reduced HIV viremia and delay in onset of disease), this method is not considered broadly applicable and manufacturing is not scalable.

It has also been suggested to prompt HIV positive patients to produce neutralizing antibodies against HIV by exposing them to viruses similar to, but less pathogenetic than, HIV. US 6,033,672 (Douvas) teaches the use of caprine arthritis-encephalitis virus (CAEV), a lentivirus found in goats with low pathogenicity in humans, for prophylactic or therapeutic purposes against HIV. CAEV capable of infecting humans have been found in people of Mexican descent, and CAEV positive individuals which develop anti-CAEV antibodies have been shown to react to both the CAEV gp135 surface glycoprotein and the HIV gap120 envelope glycoprotein to substantially neutralize the virus. Although not heterologous in nature, these neutralizing antibodies are intended to supplement the immune response.

HNabs have also been produced in animals. WO 97/02830 (Davis) teaches methods and compositions for treating HIV involving administration of neutralizing antibodies produced in goats. The goats are immunized with viral lysates. The blood of the immunized animal is then collected, and processed by standard extraction and purification methods (e.g., ammonium sulfate precipitation followed by dialysis or gel filtration) to produce an immunomodulatory composition enriched for HNabs. Davis distinguishes between the straight, untreated serum used to monitor goat antibody production in vivo from the serum composition used to treat the patient, which is a polyclonal immunoglobulin concentrate. Other filings by Davis include WO 01/60156, WO 02/07760 and US 2002/006022. All teach processing of immunized animal sera to obtain serum extracts suitable for in vivo use. The Davis method and composition have been used to treat patients outside of the United States, as widely reported in such media sources as the Washington Post (April 9, 2000) and on
Dateline Houston (September 18, 1998). This method, however, has not been shown to have the ability to lower viral load over the long term. Furthermore, the viral lysate is a single clone of laboratory virus that is known to be more susceptible than the HIV found in infected individuals, and therefore the composition has not been formulated to treat the condition as it exists in humans.

Generation of HNabs in animals permits rational design of immunogens. The HIV-1 envelope glycoprotein gp120 mediates receptor binding and is a major target for neutralizing antibodies. Purified gp120, however, has been shown to elicit type specific neutralizing antibodies, making it unsuitable for production of broadly neutralizing HNabs. Immunogen design therefore turned toward other epitopes. U.S. 6,456,172 (Gelder et al.) teaches methods and compositions for treating HIV involving administration of HNabs which recognize viral epitopes that fail to elicit neutralizing antibodies in humans when encountered through natural infection. The neutralizing antibodies are produced in goats, and antisera are processed to produce the therapeutic compositions. More specifically, the antisera is fractionated with octanoic acid, centrifuged and then filtered. The immunoglobulin fraction is then purified over a series of columns, filtered and then brought to the desired concentration of neutralizing antibody. The neutralizing antibody composition of Gelder et al. corresponds to HRG214, a polyclonal antibody preparation manufactured by Vironyx Corporation, recently the subject of a Phase I clinical trial. Dezube, BJ et al., J Infect Dis, 187(3):500-3 (2003).

Efforts at rational immunogen design for generating therapeutic HNabs are varied. See, e.g., WO 89/014941 (Oldstone) (HIV-1 related polypeptides capable of immunologically mimicking an antigenic determinant on the HIV-1 virus TMP protein); WO 91/09872 (Shaferman) (fusion proteins of highly conserved polypeptides sequences derived from gp41 and gp120 joined to non-HIV polypeptides); US 6,103,238 (Essex et al.) (deglycosylated HIV-1 envelope proteins); US 6,290,963 (Fischinger et al.) (various native and recombinant peptides). Despite the significant efforts made in this regard, it has proven extremely difficult to generate broadly cross-reactive neutralizing antibodies against HIV. Kim, YB et al., Virology. 5:305(1):124-37 (2003).

It has also been suggested to use HNabs produced in goats to treat diseases other than HIV. U.S. Patent No. 5,143,727 discloses that neuropathic diseases such as multiple sclerosis, Bechet disease, myasthenia gravis, amyotrophic lateral sclerosis, systemic lupus erythematosus, Parkinson disease, and slow virus infection can be treated with milk of an
animal such as cow or goat, preferably in her pregnancy that has been immunized by viruses. Significant anti-virus antibody are contained in the milk, particularly, colostrum and, upon oral administration of the milk the intractable disease or diseases can be treated.

Recently, it has been suggested that beneficial effects observed when these processed goat serum extracts are used to treat HIV may not be attributable to HNabs. WO 03/004049 (Dalgleish) teaches that therapeutic activity of goat serum processed in the manner of Davis et al. to provide polyclonal immunoglobulin concentrates may actually be dependent on anti-HLA and/or anti-FAS antibodies. The suggestion is made that the anti-inflammatory effect of these antibodies is beneficial in preventing the over stimulation of the immune system by viral epitopes (gp120) that resemble normal human HLA. Dalgleish further suggests a possible role for other antibodies (e.g., the dopamine receptor, the serotonin receptor, the nerve growth factor receptor p75, and the chemokine CXCL10), alone or in combination with anti-HLA and anti-FAS. Dalgleish has speculated that some observations of therapeutic benefit may be attributable at least in part to unidentified agents.

US 4,883,662 (Stout) discloses a method for treating cancer by immunizing an animal such as a goat with a normally immunosuppressive parvovirus and allowing the animal sufficient time to generate a unknown biologic thought to be interleukin. Similar to Davis and Gelder, Stout teaches processing of the serum (i.e., by ammonium sulfate fractionation) to isolate the desired biologic.

US 5,219,578 (Ansley) teaches a method of stimulating the immune system of mammals to ward off infectious disease (i.e., equine lower respiratory disease or ELRD in horses) using a fraction (i.e., the IgG) of goat sera free from foreign or artificially induced antigens. Ansley teaches that the non-immunized goat sera induce non-specific activation of the immune system in the treated animal which permits that animal to ward off infections. Ansley teaches purification of the serum by ammonium sulfate fractionation and dialysis

Given the serious epidemic of HIV infection, there exists a need for an effective means of inhibiting HIV infection that simplifies treatment regimens, increase compliance, and reduces the side effects experienced by the patient.

Therefore, it is an object of the present invention to provide a composition and method for the treatment of patients infected with HIV.

It is another object of the present invention to provide a composition and method to treat patients infected with HIV that consists of a simple regimen of therapy.
It is another object of the present invention to provide a composition and method to treat patients infected with HIV that encourages compliance with the therapy.

It is still another object of the invention to provide a composition and method to treat patients infected with HIV that has minimal side effects.

It is yet another object of the present invention to provide a composition and method to treat HIV that provides the patient with an early feeling of recovery, strength and well being.

It is still another object of the present invention to provide a composition and method to treat HIV that significantly reduces viral load a short time after initiation of therapy.

It is another object of the present invention to provide a method for the manufacture of a composition for the treatment of HIV.

It is a further object of this invention to provide intermediate compositions in the manufacture of a composition for the treatment of HIV.

Summary of the Invention

It has been discovered that a patient infected with HIV can be treated with an effective amount of a heterologous plasma or serum cocktail prepared according to the steps described in detail herein. This method provides an improvement over the prior published and practiced methods of treating HIV using purified serum extracts described in the Background. Patients treated according to this regime report one or more of an early sense of well being, a significantly improved quality of life, decreased viral loads and increased CD4 cell count. This method provides an ease of compliance that is advantageous over conventional small molecule or biologic therapy. Alternatively, patients can combine or alternate this therapy with other available therapies.

The methods of Davis and others described above use antibodies or other components isolated from animal serum by various purification techniques. The present method uses filtered, but otherwise unpurified serum or plasma of HIV-exposed animals. The term "unpurified serum or plasma cocktail" means serum or plasma as it is derived by separation from the animal blood (i.e., by centrifugation to remove the cellular elements and/or clotting), which has not be subject to any technique designed to purify, separate, isolate or concentrate any component of the serum or plasma. This includes crude protein separation by such
techniques as ammonium sulfate precipitation. It has been surprisingly discovered that components of unpurified serum or plasma are beneficial to patient treatment as indicated by the significant and early patient response. Without being bound by any particular theory, it is believed that the unpurified serum or plasma retains a beneficial plethoric effect generated in vivo. This beneficial plethoric effect is thought to involve the presence of a mixture of components in the animal serum or plasma. Preliminary data, as shown in Example 6, suggest that although antibodies may contribute to the observed effect, they are not likely responsible for the therapeutic benefits. The goal of the present method is to retain as many components of the immunized goat serum as possible.

The heterologous plasma cocktail is useful in the prevention and treatment of HIV infections and other related conditions such as AIDS-related complex (ARC), persistent generalized lymphadenopathy (PGL), AIDS-related neurological conditions, anti-HIV antibody positive and HIV-positive conditions, Kaposi's sarcoma and other cutaneous lesions, thrombocytopenia purpura, Pneumocystis carinii, Mycobacterium avium, cytomegalovirus retinitis, oropharyngeal and esophageal candidiasis and other opportunistic infections as well as general anorexia, weight loss and cachexia. In addition, these compounds or formulations can be used prophylactically to prevent or retard the progression of clinical illness in individuals who are anti-HIV antibody or HIV-antigen positive or who have been exposed to HIV.

Accordingly, one aspect of the present invention is a composition that includes optionally sterilized but otherwise unpurified serum or plasma cocktail derived from a mammal exposed to HIV. Other aspects of the invention feature methods of preparation of the heterologous serum or plasma cocktail, and methods of treating or preventing HIV/AIDS utilizing the heterologous serum or plasma cocktail.

In one embodiment of the present invention, the composition is a heterologous plasma cocktail.

In another embodiment of the present invention, the composition is a heterologous serum cocktail.

The method of preparing the composition of the present invention also presents advantages over the known methods. Davis teaches the injection of preferably attenuated HIV subcutaneously or intramuscularly to over twenty sites on the animal once weekly for four weeks and then the repetition of the inoculation step at six weeks. The innoculant used to generate the heterologous serum or plasma cocktail may vary. In one embodiment, the
animal is inoculated with the blood, plasma or serum of an HIV positive individual. In a preferred embodiment, HIV is provided in the form of blood, plasma or serum from an HIV positive individual with a viral load greater than 2,000. Attenuated or heat treated virus can be used but is not preferred.

In a particular embodiment, the animal is inoculated with the plasma of an HIV positive individual exhibiting clinical manifestations of AIDS (i.e., one or more target opportunistic infections) as a means to increase the protective effect of the resulting plasma for therapeutic treatment. In another embodiment, HIV is provided in the form of blood, plasma or serum from an HIV-positive patient who exhibits the same profile or opportunistic infections as the HIV to be treated.

In another embodiment, blood from the HIV positive individual to be treated with the method of the present invention is used to produce the heterologous plasma cocktail.

According to the present method, the animal can be injected once (or more if desired), preferably subcutaneously, with the plasma of an HIV infected human or live virus, and the blood drawn after a sufficient time for the animal to respond to the injection, preferably approximately one, two or three weeks or more. In one embodiment, as little as 2-3 mls of the HIV-positive plasma is injected into the animal to produce the response.

A number of human patients have been experimentally treated with this heterologous plasma cocktail. All of the patients had previously failed at least two years of standard FDA-approved anti-HIV medication programs. Many of these patients were told nothing more could be done for them, and several were sent home for hospice care. All patients had viral loads in excess of 50,000 copies per mL, many with over 1,000,000 copies per mL, and at least one with over 9,000,000 copies per mL. U.S. treatment guidelines suggest anyone with a viral load over 55,000 should initiate treatment for HIV infection. For prognosis, viral load can help predict how long someone will stay healthy. The higher the viral load, the faster HIV progresses.

In the patients treated experimentally over a three year period to determine efficacy, one or more of the following positive outcomes have been observed.

Reduced viral load: In many patients complying with the treatment guidelines, viral loads were reduced to zero or minimal copies per mL in an average of 90 days. One patient exhibited a viral load decrease from 9,400,000 copies per mL to 1,100,000 copies per mL in thirty days.
Increased T-cell count: In patients complying with the treatment guidelines, the therapy has produced a steady increase in the T-cell count.

Immediate decreases in some HIV-related symptoms: Most patients complying with the treatment guidelines reported significant increases in energy level, appetite, sex drive, relief from chronic fatigue, and complete termination of diarrhea and nausea within 24 hours of the initial treatment. Many patients reported significant weight gains during the months following treatment, in some cases by as much as 50 lbs.

Long-term decreases in some HIV-related symptoms: With extended use of the heterologous plasma cocktail, patients experienced decreased dementia, decreased peripheral neuropathy, decreased incidence of opportunistic infections, and improvements in skin lesions, rashes, and Kaposi's Sarcoma.

No significant side effects: In the patients treated to date, the only side effects patients have reported are slight insomnia and localized skin irritation at the injection site that clears within a few minutes to several hours.

Efficacy across viral strains: In the patients treated to date, clinical data has established that the treatment is equally efficacious across each of the mutated strains of HIV found worldwide, including the wild strain.

In one aspect of the invention, therefore a process for the preparation of a heterologous plasma cocktail is provided comprising the steps of:

a) exposing a mammal not susceptible to infection to HIV;

b) allowing time for the mammal to respond to the virus and to produce one or more beneficial biologic agents in the blood; and

c) obtaining plasma which may be used straight, sterilized or dehydrated but not otherwise purified to treat or prevent HIV infection in humans.

In a particular embodiment, the plasma is obtained by:

a) collecting the blood from the mammal;

b) treating the blood with an anticoagulant to prevent the formation of serum; and

c) separating the plasma from the blood.

In a particularly preferred embodiment, the plasma is separated from the blood by centrifugation.
In another embodiment of the present invention, the plasma cocktail is sterilized by filtration.

In a particular embodiment of the present invention, the mammal is an ungulate. In one embodiment of the present invention, the mammal is a goat.

In an alternate embodiment of the invention, the heterologous serum cocktail is obtained by a process that includes the following:

a) exposing a mammal not susceptible to infection to HIV;

b) allowing time for the mammal to respond to the virus and to produce one or more beneficial biologic agents in the blood; and

c) obtaining the serum which can be used straight, concentrated, dehydrated or sterilized but not otherwise purified to treat or prevent HIV infection in humans.

In one embodiment, the serum is sterilized by filtration.

In a preferred embodiment, the serum is obtained by:

a) collecting the blood from the mammal;

b) clotting the blood; and

c) separating the clot from the serum.

In a particularly preferred embodiment, a clotting activator is added to facilitate the clotting of the blood. In another embodiment of the present invention, the blood is clotted by contact activation. In a preferred embodiment of the invention, the clot is separated from the serum by centrifugation.

In another embodiment of the present invention, there is a heterologous plasma or serum cocktail useful in the treatment of HIV which is prepared by:

a) exposing a mammal not susceptible to infection to HIV;

b) allowing time for the mammal to respond to the virus and to produce one or more beneficial biologic agents in the blood; and

c) obtaining plasma or serum which may be used straight or sterilized but not otherwise purified to treat or prevent HIV infection in humans.

In another embodiment of the present invention, there is a unpurified heterologous plasma or serum cocktail useful in the treatment or prevention of HIV.
In another embodiment of the present invention, there is a sterilized but otherwise unpurified heterologous plasma or serum cocktail useful in the treatment or prevention of HIV.

In another particular embodiment of the invention, the status of the HIV-exposed mammal may be monitored after exposure to HIV to determine if sufficient beneficial levels of one or more biological factors have been produced. The exposed animal’s blood, plasma or serum is drawn and tested in vitro for the ability to inhibit HIV infection. Once inhibition is observed, blood can be drawn to generate the plasma or serum cocktail as described above.

The plasma or serum cocktail of the present invention can be frozen prior to use in humans.

In another aspect of the invention, a composition comprising an effective anti-HIV amount of a heterologous plasma cocktail for the treatment and/or prophylaxis of an HIV infection in a host, such as a human, optionally with a pharmaceutically acceptable carrier or diluant is provided by carrying out the steps described above.

In another aspect of the invention, use of an effective anti-HIV amount of heterologous plasma cocktail for in combination or alteration with an anti-HIV agent the treatment and/or prophylaxis of an HIV infection in a host, such as a human, is provided.

**Brief Description of the Drawings**

Figure 1 is a graphical presentation of inhibition of HIV-1 viral strain BR/92/014 replication in PHA-stimulated human peripheral blood mononuclear cells (PBMCs) by a preparation, VR-30 (HV), of the goat plasma prepared as described in Example 3 of the present invention.

Figure 2 is a graphical presentation of inhibition of HIV-1 viral strain HT/92/599 replication in PHA-stimulated human peripheral blood mononuclear cells (PBMCs) by a preparation of the goat-derived plasma cocktail of the present invention, VR-30 (TV).

Figure 3 is a graphical presentation of inhibition of HIV-1 viral strain HT/92/596 replication in PHA-stimulated human peripheral blood mononuclear cells (PBMCs) by a preparation of the goat-derived plasma cocktail of the present invention, VR-30 (TV).
Detailed Description of the Invention

It has been discovered that a patient infected with HIV can be treated with an effective amount of a heterologous plasma or serum cocktail prepared according to the steps described in detail herein. This cocktail can also be used as a prophylaxis.

The heterologous plasma or serum cocktail has been experimentally administered to a number of HIV-infected patients, resulting in one or more of substantially reduced viral load down to minimal in an average of 90 days, increases T-cell count, production of immediate decreases in HIV symptoms, such as fatigue, diarrhea, nausea, low appetite, and low sex drive, long-term decreases in HIV symptoms, such as dementia, peripheral neuropathy, opportunistic infections, skin lesions, and Kaposi’s sarcoma, does not produce significant side effects, provides efficacy across all strains, including the wild strain.

In particular, the invention includes at least the following embodiments:

(1) A process for preparing a heterologous plasma cocktail which can be used to treat a host infected with human immunodeficiency virus (HIV), which comprises:

a) exposing an animal to HIV which does not exhibit infection on such exposure;

b) allowing time for the animal to respond to the virus and to produce one or more beneficial biologic agents in the blood;

c) obtaining plasma from the animal; and

d) using the plasma itself, with optional sterilization, dehydration, dilution or pharmaceutical preparation, but without further purification, to provide the heterologous plasma cocktail.

(2) The process of (1) described above, wherein the animal is a mammal.

(3) The process of (1) described above, in which the plasma is obtained by: collecting the blood from the animal, treating the blood with an anticoagulant to prevent the formation of serum; and separating the plasma from the blood.

(4) The process of (3), in which the plasma is separated from the blood by centrifugation.

(5) The process of (1), in which the plasma cocktail is sterilized by filtration.

(6) A process for preparing a heterologous serum cocktail which can be used to treat a host infected with human immunodeficiency virus (HIV), which comprises:
a) exposing an animal to HIV which does not exhibit infection on such exposure;

b) allowing time for the animal to respond to the virus and to produce one or more beneficial biologic agents in the blood;

c) obtaining serum from the animal; and

d) using the serum itself, with optional sterilization, dehydration, dilution or pharmaceutical preparation, but without further purification, to provide the heterologous serum cocktail.

(7) The process of (6), in which the serum is obtained by: collecting the blood from the mammal; clotting the blood or allowing the blood to clot; and separating the clot from the serum.

(8). The process of (7), wherein the clotting factor is selected from the group consisting of heparin, EDTA, citrate or oxalate.

(9). The process of (6), wherein the serum is clotting by contact activation.

(10). A pharmaceutical composition useful in the treatment of HIV which is prepared by:

a) exposing an animal not susceptible to infection to HIV;

b) allowing time for the animal to respond to the virus and to produce one or more beneficial biologic agents in the blood;

c) obtaining the plasma from the animal; and

d) providing the plasma itself, with optional sterilization, dehydration, dilution or pharmaceutical preparation, but without purification, as the pharmaceutical composition for administration.

(11). The composition of (10) which is packaged as a pharmaceutical product.

(12) The composition of (10) which is prepared as an intravenous formulation, an injectable formulation, a parenteral formulation, an oral formulation or an intraarterial formulation.

(13). The composition of (10), wherein the cocktail is sterilized by filtration.

(14) The composition of (10), comprising a pharmaceutically acceptable carrier or a diluent to the plasma.
(15). A pharmaceutical composition useful in the treatment or prevention of HIV comprising unpurified plasma or serum from a goat immunized with HIV.

(16). The composition of (15), wherein the goat plasma or serum which has been sterilized by filtration.

(17). A process for preparing a heterologous plasma or serum cocktail which can be used to treat a host infected with human immunodeficiency virus (HIV), which comprises:
   a) exposing an animal to HIV which does not exhibit infection on such exposure;
   b) allowing time for the animal to respond to the virus and to produce one or more beneficial biologic agents in the blood;
   c) obtaining blood from the mammal;
   d) monitoring the blood for sufficient beneficial levels of one or more biological factors; and
   e) obtaining plasma or serum, with optional sterilization, dehydration, dilution or pharmaceutical preparation, but without further purification, to provide the heterologous plasma or serum cocktail.

(18). The process of (17), in which the blood is monitored for the ability to inhibit HIV infection in vitro.

(19). A method for the treatment of a patient infected with HIV comprising administering an effective amount of the heterologous plasma cocktail as described above.

(20). A pharmaceutical composition useful in the treatment of HIV which is prepared by:
   a) exposing an animal not susceptible to infection to HIV;
   b) allowing time for the mammal to respond to the virus and to produce one or more beneficial biologic agents in the blood;
   c) obtaining the serum from the animal; and
   d) providing the serum itself, with optional sterilization, dehydration, dilution or pharmaceutical preparation, but without further purification, as the pharmaceutical composition for human administration.

(21). The heterologous cocktail as described above for use in medical therapy.
(22). The heterologous cocktail as described above for use in the treatment of a human infected with HIV.

(23). The use of the heterologous cocktail as described above for use in the manufacture of a medicament for the treatment of a patient infected with HIV.

(24). A process for preparing a heterologous plasma or serum cocktail which can be used to treat a host infected with human immunodeficiency virus (HIV), which comprises:
a) obtaining plasma or serum from an animal not susceptible to infection to HIV; and
b) using the plasma or serum itself, with optional sterilization, dehydration, dilution or pharmaceutical preparation, but without further purification, to provide the heterologous plasma cocktail.

(25). The process of (24), wherein the animal is a goat.

(26). A pharmaceutical composition comprising the cocktail of (24) in combination with a pharmaceutically acceptable carrier.

Process for Producing the Plasma Cocktail

The following are nonlimiting embodiments of how to carry out the invention. Given the description of the invention provided throughout this text, one of ordinary skill can modify the steps below without deviating form the spirit or scope of the invention.

(i) Selection of animal

The heterologous plasma or serum cocktail may be prepared using any animal or mammal not susceptible to infection with HIV that produces an effective product on inoculation according the process described in detail herein. Non-limiting examples of animals suitable for this purpose include cow, rabbit, cat, dog, mouse, goat, lamb, sheep, horse, deer, pig, mouse, chicken and the like (for example Bora goats). In a particular embodiment, the mammal is an ungulate or hooved-mammal. Non-limiting examples of ungulates include goats, sheep, horses, and cows. In a particularly preferred embodiment, the mammal is a goat.

(ii) Inoculation of the animals

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Any HIV-bearing inoculates suitable for generating the heterologous plasma or serum cocktail can be used in the present invention. Non-limiting examples include the blood, plasma or serum of an HIV positive individual, a viral lysate, naturally occurring or synthetic viral proteins or peptides (glycosylated or unglycosylated). In a particular embodiment, the inoculant is plasma derived from an HIV positive individual. It is preferred that the HIV positive blood, plasma or serum used as the inoculant come from an HIV positive individual with a viral load greater than 2,000.

Plasma from an HIV positive individual manifesting clinical symptoms of HIV (i.e., opportunistic infections) the same or different than the clinical symptoms of the patient to be treated can be used as the inoculant.

In a particular embodiment, the plasma of the HIV POSITIVE infected individual to be treated according to the present invention is used as the inoculant.

Attenuated or heat treated virus can be used but is not preferred.

According to one embodiment of the present invention, human blood is drawn from an HIV positive patient using standard, sterile, phlebotic techniques. Preferably, the HIV positive donors are between 18 and 65 years of age. Preferably, donors should appear healthy, not be under the influence of drugs or alcohol, and weigh in excess of 50 kg (110 lb). The following criteria of health can also be useful: body temperature less than 37.5 °C; pulse regular (50 to 100 beats per minute); blood pressure lower than 180 mm Hg systolic and 100 mm Hg diastolic; hemoglobin greater than 12.5 g/l and hematocrit greater than 38%. The blood is then processed to produce plasma according to techniques well known to those skilled in the art.

The plasma obtained from an HIV positive individual can then be used to inoculate a mammal, such as a goat. The plasma can be injected one or more times. In addition, various adjuvants can be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (Bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are also well known in the art. Additional non-limiting examples of adjuvants suitable for use in the present invention are discussed further below.
The plasma and/or adjuvant can be injected in the mammal by one or more subcutaneous or intraperitoneal injections, though they can also be given intramuscularly, and/or intravenously.

The mammal can be given a sedative, for example Rompun, to facilitate handling of the mammal if necessary.

At least 1 cc of human plasma can be administered to the mammal. Preferably, between 1-10 cc of the plasma can be administered to the animal subcutaneously. Alternatively, at least 1, 2, 5, 7, 10, 15, 20, 25, 30, 40 or 50 cc of plasma is administered subcutaneously, intraperitoneally intramuscularly, and/or intravenously.

(iii) Monitoring of animal

The animal should preferably be monitored to indicate the patient sample with which it was injected and the date of injection and the animal should be monitored over a time period, ranging from at least 1 to at least 8 weeks. Blood samples can be obtained from the animal during this time to measure the generated immune response. For example, the plasma from the blood sample can be measured for the ability of the plasma to inhibit HIV infection of CD4 cells in vitro. The mammal can be a goat; it is known that in goats three weeks is the standard period of incubation for generating a sufficient immune response.

To determine whether useful HIV antibodies are produced (although such may not be necessary to the efficacy of the cocktail product), one can assess the material using the procedures of various techniques are known in the art that include, but are not limited to: HIV-1 ELISA, HIV-1 Western blot, HIV-2 ELISA, HTLV I/II ELISA, HTLV I/II Western Blot, HIV-1 p24 Antigen Capture, HIV-1 Culture (with p24 Antigen Capture), DNA-PCR for Detection of HIV-1/2 and HTLV I/II, Roche AmplicorTM Quantitative RNA-PCR (HIV-1 Viral-Burden/Viral Load). Other types of immunoassays known in the art can be used, including but not limited to, competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunabsorbant assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, in situ immunoassays,(using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc.
An ELISA is a technique that uses antigens to coat the well of plates. ELISAs involve coating the well of a multiwell, such as a 96-well, microtiter plate with the antigen, washing away antigen that did not bind the wells, adding the blood or blood product from the mammal that has been inoculated conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) to the wells and incubating for a period of time, washing away unbound or non specifically bound materials, and detecting the presence of the specifically bound blood or blood product to the antigen coating the well. Alternately, in ELISAs the blood or blood product from the mammal that has been inoculated does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes the blood or component of the blood) can be conjugated to a detectable compound and added to the well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, e.g., Ausubel et al, eds, 1994, 10 Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York Bollag, D.M., Rozycki, M.D., and Edelstein, S.J. (1996). Protein Methods, Second Edition. New York: Wiley-Liss, 195-227.

For example, an HIV-1 ELISA test detects circulating antibodies to HIV 1 virus in blood or serum. This test utilizes a whole viral lysate ELISA and is performed on either serum or plasma. An HIV-2 ELISA is a similar test that detects circulating antibodies to HIV 2 virus. The ELISAs are FDA-approved for both donor screening and diagnostic use. Versions of these kits are commercially available (Biorad TM).

Another example is an HTLV I/II ELISA is used to detect antibodies against HTLV I/II viruses. A commercially available kit is available, for example, from Organon TeknikaTM. This test is performed on either serum or plasma. The kit is FDA-approved for both diagnostic use and/or screening.

A further example is an HIV-1 p24 Antigen Capture is a FDA-approved test performed on either serum or plasma to detect the viral protein p24. After acid dissociation of immune complexes, the ELISA is performed using mouse antibody to detect the HIV-1 p24 protein.

Another useful technique is a western blot analysis. Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (e.g., 8%- 20% SDS-PAGE depending on the molecular weight of the
antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (e.g., PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (e.g., PBS-Tween 20), applying the proposed binding protein (diluted in blocking buffer) to the membrane, washing the membrane in washing buffer, applying a secondary antibody (which recognizes the HIV protein that you are assaying for) conjugated to an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) or radioactive molecule (e.g. $^{125}$I) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, e.g., Ausbel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc, New York 30; Harlow, E. and Lane, D. (1988). Antibodies: A Laboratory Manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 471-510.

For example, an HIV-1 western blot test is performed on either serum or plasma to detect and identify antibodies against the HIV-1 retrovirus. A commercially available kit is available from Cambridge Biotech TM for HIV-1 confirmation testing. This kit is FDA-approved for both donor screening and confirmatory diagnostic use.

In another example, an HTLV I/II Western Blot can also be performed. The test can be performed on either serum or plasma to detect antibodies against HTLV I/II viruses. Commercially available kits are available, for example, one is supplied by Cellular ProductsTM.

Another method is the HIV-1 Culture (with p24 Antigen Capture) test is performed on whole blood to detect the HIV-1 retrovirus. This test is may be particularly useful in determining antibody levels that might go undetected with other testing procedures and kits.

A further method is DNA-PCR for Detection of HIV-1/2 and HTLV I/II is a method of detecting HIV-1, HIV-2, HTLV I or II viruses using PCR, an enzymatic reaction amplifying specific segments of the retroviral genome in material harvested from blood cells. Detection and identification of a retrovirus depends upon the virus-specific primers (DNA oligomers) used and which may be provided by a reference laboratory.

In addition, quantitative RNA-PCR (HIV-1 Viral Burden/Viral Load) is another method whereby viral RNA is quantified. This enzymatic reaction amplifies a specific region
of the viral RNA extracted from non-heparinized plasma. Viral RNA levels between 400 - 750,000 copies/ml are quantitated using the standard assay, whereas the ultrasensitive procedure quantitates viral RNA levels at 50-75,000 RNA copies/ml. This test has been FDA approved for patient management uses. Commercial kits are available, such as the Roche Amplicor HIV-1 MonitorTM standard assay (v1.0).

Other techniques for evaluating immune responses in mammals are known to one skilled in the art.

(iv) Removal of blood from inoculated animal

To obtain the plasma from the mammal, blood has to be collected. Any means to do this which accomplishes the desired goal is suitable. It is preferable to obtain large quantities of blood from the mammal, for example 10-30 cc of blood from a rabbit or similar sized animal and higher quantities from larger animals. The blood should begin to flow immediately through the tubing to the syringe, vacutainer, or open tube/bottle. If a syringe is used, gently draw on the syringe to collect the blood, and once the syringe is full, change syringes by disconnecting from the infusion set or needle hub. (See, for example, McGuill, M.W. and Rowan, A.N., "Biological Effects of Blood Loss: Implications for Sampling Volumes and Techniques," ILAR News, Vol. 31(4), Fall 1989, pp 5-20).

In a particular embodiment of the present invention, the blood is collected in a manner that prevents coagulation in order to obtain plasma not serum. A variety of methods are known in the art for preventing coagulation of drawn blood, and include, without limitation, collecting the blood in tubes or other types of collecting means that have been treated with an anticoagulant. Anticoagulant coated test tubes of this type are widely available commercially. Suitable anticoagulants include, but are not limited to EDTA, heparin, citrate or oxalate. Tube inversions allow proper mixing of anticoagulant additives and blood.

Alternatively, a syringe and the infusion set tubing used in harvesting the blood can be filled with anticoagulant to aid in the harvesting of the plasma. Alternatively, the blood can be collected into a vacutainer or bottle that has been treated with anticoagulant. Alternatively, anticoagulants can be added to the plasma component of the blood after the cellular elements have been removed, for example by centrifugation as described below.

It is has been observed that plasma may not remain anticoagulated over time (i.e., it may clot to produce serum) unless proper techniques are utilized. Techniques for preventing plasma instability are known to those skilled in the art.
In one embodiment of the present invention, the composition is a heterologous serum cocktail. When serum is desired, as opposed to plasma, the blood should be collected in a way that permits coagulation. Blood will naturally coagulate in a collection tube as coagulation factors become activated upon contact with a negative surface ("contact activation"). The time required to clot plasma may vary, and may range from less than a minute to more than an hour. Alternatively, the clotting process can be accelerated by the addition of a clotting activator to the tube or other container used to collect blood. Non-limiting examples of suitable clotting activators include calcium or silica particles.

The animal can be sedated. For example, 0.5cc Rompun can be used to sedate, for example, a goat. In another example, Torbugesic (butorphanol; 1 mg/kg) and acepromazine maleate (1 mg/kg) can be used to sedate, for example, a rabbit. After the animal is sedated, the blood can be collected. One way to remove blood from an animal is to cannulate an artery, for example the external jugular artery. The mammal can be a goat and for a goat, an at least 18 gauge needle can be used to extract at least 150 cc of blood, preferable between 200 - 400cc of blood. In another example, a needle, at least 21 gauge, is connected to an infusion device, such as an E-Z infusion set, to a syringe, for example, at least a 10 or 20 cc syringe.

As the blood is collected, it is preferably stored in a cooled environment, for example on ice or in a refrigerator or freezer.

Treatment of conditioned blood to form the heterologous plasma or serum cocktail

The following description describes one way in which the blood can be treated to form the heterologous plasma or serum cocktail.

In one embodiment, plasma is separated from blood by centrifugation. Centrifugation speeds and times are known to those skilled in the art, and may depend, for example, upon the type of tube used for blood collection. The specific gravity ranges for red cells are sufficiently different to enable isolation by centrifugation. Plasma is then obtained from the appropriate fragment.

In one embodiment of the present invention, the plasma can be repeatedly centrifuged to minimize the number of residual cells in the plasma fraction.

In another embodiment, serum is separated from clotted blood by centrifugation. Certain types of tubes known to those in the art may facilitate the separation process. For
example, tubes containing a gel substance such that when the tube is centrifuged the cells go below the gel while the serum remains above.

Transmission of infectious disease (i.e., by viruses, bacteria or parasites) remains a concern in the use of any blood or blood product such as plasma or serum. In a further embodiment of the present invention, the blood or plasma can be sterilized prior to in vivo use. Any suitable method can be used to achieve sterilization as long as the method does not alter the product in such a way as to diminish its efficacy. Non-limiting examples of sterilization techniques suitable for use with the present invention include chemicals, heat, ultraviolet radiation and photosensitizing dyes. The plasma can also be filtered to achieve sterilization. Recent advances and new strategies for the inactivation and removal of infectious agents are contemplated for use in the present invention.

In one embodiment of the present invention, the plasma is repeatedly centrifuged and filtered. For example, the plasma is spun at approximately 32,000 rpm on a standard centrifuge. The resultant supernatant can then be transferred, preferably under sterile conditions using sterile techniques, and then suction filtered through a 0.5 micron filter. During this preparation, the sample can be kept on ice between the centrifugation and filtration steps. The plasma can then be passed over a filter, for example a filter with at least 0.2 micron pores, and then placed in an ultracentrifuge, preferably non-refrigerated, to spin at approximately 90,000 rpm for at least 20 minutes. The supernatant can then be placed in containers, preferably sterile, in an ultracentrifuge, preferably non-refrigerated, to spin at at least 150,000 rpm for at least 20 minutes. After the centrifugation, the supernatant can be passed through an anhydrous filter. The plasma can be repeatedly filtered, preferably through a 0.2 micron filter and a smaller filter, such as a 0.1 micron filter. Passage through a 0.1 micron filter allows for the plasma to be deemed sterile.

(vi) Storage and testing of heterologous plasma or serum cocktail

The resulting plasma or serum preparation can be placed in small aliquots (e.g., between 2 - 10cc each) and stored for later use. Proper storage conditions for plasma and serum with respect to temperature and time are well known to those skilled in the art. For example, test tubes containing small aliquots of the heterologous plasma or serum cocktail can be stored at -70°C, for at least 48 hours.

After a suitable time has passed for the samples to be stored, such as 48 hours, individual aliquots can be brought to room temperature for sterility testing. For example, the
sample can be cultured under both anaerobic and aerobic conditions to test for contamination. If the cultures are negative, the remaining aliquots of can then be administered to a patient.

(vii) Administration of heterologous plasma or serum cocktail to patient in need thereof

The heterologous plasma or serum cocktail can be administered to a patient in need thereof through any means provided in this application (See Pharmaceutical Compositions below). In one embodiment, the patient can receive a therapeutically effective dosage, preferably between 2 - 10cc if administered subcutaneously, and treatment duration can vary based on the severity of the HIV infection. For example, treatment can range from daily administration for a patient with a CD4 count of less than 400 to a single, unrepeated dose administered, for example, to an HIV positive still asymptomatic patient.

Anti-HIV Agents That Can Be Used in Combination and/or Alternation With the Composition of the Present Invention

The heterologous plasma cocktail can be administered alone or can be administered in combination or alternation with another anti-HIV agent or agent that treats a concomitant opportunistic infection. In general, during alternation therapy, an effective dosage of each agent is administered serially, whereas in combination therapy, effective dosages of two or more agents are administered together. The dosages will depend on such factors as absorption, bio-distribution, metabolism and excretion rates for each drug as well as other factors known to those of skill in the art. It is to be noted that dosage values will also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens and schedules should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions. Examples of suitable dosage ranges can be found in the scientific literature and in the Physicians Desk Reference. Many examples of suitable dosage ranges for other compounds described herein are also found in public literature or can be identified using known procedures. These dosage ranges can be modified as desired to achieve a desired result.

The disclosed combination and alternation regiments are useful in the prevention and treatment of HIV infections and other related conditions such as AIDS-related complex (ARC), persistent generalized lymphadenopathy (PGL), AIDS-related neurological conditions, anti-HIV antibody positive and HIV-positive conditions, Kaposi's sarcoma,
thrombocytopenia purpura and opportunistic infections. In addition, these compounds or formulations can be used prophylactically to prevent or retard the progression of clinical illness in individuals who are anti-HIV antibody or HIV-antigen positive or who have been exposed to HIV.

As non-limiting examples, the heterologous plasma cocktail can be administered in combination or alternation with any of the following currently approved drugs.

- **Abacavir** (Ziagen), Glaxo Wellcome
  Approved to treat HIV-1 in adults and children.

- **Agenerase** (Amprenavir) Glaxo Wellcome
  Approved to treat HIV-1 in adults and children.

- **Aldesleukin** (Proleukin), Chiron Corp
  Currently in Phase I/II trials for treatment of HIV

- **Alitretinoin** (Panretin) gel 0.1%, Ligand Pharmaceuticals
  Approved for the topical treatment of cutaneous lesions in patients with AIDS-related Kaposi's sarcoma.

- **AMD11070**, Anormed Pharmaceuticals
  Currently in Phase I clinical trials for the treatment of HIV.

- **Amphotericin B Liquid Complex** (Abelcet, ABLC, Ambisome), The Liposome Company
  Approved for the treatment of aspergillosis

- **Atovaquone** (Mepron, 566C80), Glaxo Wellcome
  Approved for the treatment of mild to moderate *Pneumocystis carinii* pneumonia (PCP) in patients who are intolerant to Bactrim or Septra.
  Approved for the treatment of mild to moderate PCP in patients who are intolerant of Trimethoprim-Sulfamethoxazole (TMP-SMX)
  Approved for the prevention of PCP.

- **Atazanavir** (Reyataz), Bristol-Myers Squibb,

- **Azithromycin** (Zithromax), Pfizer, Inc.
  Approved for the prevention of *Mycobacterium avium* complex in persons with advanced HIV infection.

- **Beta-L-FddC**, Achillion Pharmaceuticals
  Currently in Phase II clinical trials for treatment of HIV.
• BMS 561390, Bristol-Myers Squibb
  Currently in Phase II clinical trials for treatment of HIV.

• Calanolide A, Sarawak Medicam Pharmaceuticals
  Currently in Phase II clinical trials for the treatment of HIV.

• Capravirine, Pfizer and Agouron Pharmaceuticals
  Currently in Phase II clinical trials for the treatment of HIV.

• CCR5 Receptor Antagonist, Schering Plough
  Currently in Phase I clinical trials of HIV.

• CD4-IgG2 (PRO 542), Progenics Pharmaceuticals
  Currently in Phase II clinical trials for treatment of HIV.

• Cidofovir (Vistide, HP MPC), Gilead Sciences, Inc.
  Approved for the treatment of AIDS-related cytomegalovirus retinitis.

• Clarithromycin (Biaxin, Klacid), Abbott Laboratories
  Approved for the treatment of disseminated mycobacterial infections due to
  Mycobacterium avium-intracellulare complex (MAC). Approved for prophylaxis of
  disseminated MAC in patients with advanced HIV infection.

• Cytolin A, Amerimmune Pharmaceuticals
  Currently in Phase I/II clinical trials for treatment of HIV.

• DAPD (Amodoxovir), Gilead Sciences
  Currently in Phase II clinical trials for treatment of HIV.

• Daunorubicin-liposomal (DaunoXome), Nexstar
  Approved April 8, 1996 for the treatment of advanced HIV-related Kaposi's sarcoma.

• Delavirdine mesylate (DLV, Rescriptor), Pharmacia & Upjohn
  Approved for use in combination with appropriate antiretrovirals when therapy is
  warranted for treatment of HIV infection.

• Didanosine (ddI, Dideoxyinosine, Videx), Bristol Myers-Squibb
  Approved for treatment of adult and pediatric patients with advanced HIV who are
  intolerant to or deteriorating on AZT.
  Expanded indication and dosage recommendations reduced.
  Approved for treatment of HIV infection when antiretroviral therapy is warranted.
  Approved Videx EC, enteric coated capsule, approved for combination with other
  antiretroviral agents, as indicated for the treatment of HIV-1 infection in adults whose
management requires once-daily administration of didanosine or an alternative didanosine formulation.

- **Doxorubicin hydrochloride-liposomal** (Doxil), Sequus Pharmaceuticals, Inc. Approved for the treatment of Kaposi's sarcoma in AIDS patients who are intolerant to or have disease progression on prior combination chemotherapy.

- **Dronabinol** (Marinol), Roxane Laboratories Approved for the treatment of anorexia associated with weight loss in patients with AIDS.

- **Efavirenz** (Sustiva), DuPont Pharmaceuticals Approved for the treatment of HIV-1 infection, in combination with other antiretroviral agent(s). New Drug Application

- **Emtriva** (FTC), Gilead Sciences, Inc. Approved for the treatment of HIV

- **Enfuvirtide** (Fuzeon, T 20), Roche and Trimeris Approved for the treatment of HIV

- **Erythropoietin** (EPO, Epogen, Procrit), Amgen Approved for the treatment of anemia related to AZT therapy in HIV infection.

- **Famciclovir** (Famvir), SmithKline Beecham Approved for the treatment of recurrent mucocutaneous herpes simplex infections in HIV-infected patients.

- **Fluconazole** (Diflucan), Pfizer, Inc. Approved for the treatment of oropharyngeal and esophageal candidiasis and for the treatment of cryptococcal meningitis. Approved for the treatment of pediatric patients with cryptococcal meningitis and candida infections.

- **Fomivirsen Sodium Injection** (Vitravene intravitreal injectable), Isis Pharmaceuticals Approved for the local treatment of cytomegalovirus (CMV) retinitis in patients with acquired immunodeficiency syndrome (AIDS) who are intolerant of or have a contraindication to other treatment(s) for CMV retinitis or who were insufficiently responsive to previous treatment(s) for CMV retinitis.

- **Fosamprenavir** (GW433908, VX-175), Vertex and Glaxo Wellcome Currently in Phase III clinical trials for treatment of HIV.
• **Foscarnet** (Foscavir), Astra Pharmaceuticals
  Approved for the treatment of cytomegalovirus (CMV) retinitis in patients with AIDS.
  Approved for the treatment of acyclovir-resistant mucocutaneous herpes simplex virus infections in immunocompromised patients.
  Approved for combination therapy with foscavir and ganciclovir of patients who have relapsed after monotherapy with either drug.

• **Ganciclovir** (IV, Oral)(Cytovene, DHPG), Syntex
  Approved (IV) for treatment of cytomegalovirus (CMV) retinitis in immunocompromised patients.
  Approved (oral) for maintenance therapy for CMV retinitis in some patients.
  Approved (oral) for prophylaxis of CMV disease.

• **Ganciclovir** (Implant) (Vitraser), Chiron Corporation
  Approved for the treatment of cytomegalovirus retinitis in patients with AIDS.

• **Gem 92**, Hybirdon
  Currently in Phase I clinical trials for treatment of HIV.

• **GS-7340-02**, Gilead Sciences
  Currently in Phase I/II clinical trials for treatment of HIV.

• **Immune globulin** (IV) (Gamimmune N, Gamma Globulin, IGIV), Bayer Pharmaceutical Division
  Approved for prevention of bacterial infections in pediatric HIV infection.

• **Indinavir sulfate** (Crixivan, IDV, MK-639), Merck & Co.
  Approved for use alone or in combination with nucleoside analogues for the treatment of HIV infection in adults. Accelerated Approval
  Approved new 333 mg capsule formulation

• **Interferon Alfa-2a** (Roferon-A), Hoffman-La-Roche
  Approved for the treatment of AIDS-related Kaposi’s sarcoma in selected patients.

• **Interferon Alfa2b** (Intron-A), Schering-Plough
  Approved for the treatment of adult AIDS-related Kaposi’s sarcoma.
  Approved for the treatment of chronic hepatitis C (Non-A, Non-B hepatitis or HCV).

• **Interleukin-2 SA** (Bay 50-4798), Bayer Pharmaceutical
  Currently in Phase I/II clinical trials for treatment of HIV.
• **Itraconazole** (Sporanox), Janssen Pharmaceutical
  Approved for the treatment of histoplasmosis, blastomycosis, and aspergillosis in immunocompromized and non-immunocompromised patients.
  Approved for the treatment of pulmonary and extra pulmonary aspergillosis in patients who are intolerant of or refractory to amphotericin B.
  Approved (oral solution) for the treatment of oropharyngeal and esophageal candidiasis.

• **Kaletra** (lopinavir and ritonavir), Abbott Laboratories
  Approved for combination with other antiretroviral agents for the treatment of HIV-1 infection in adults and pediatric patients age six months and older.

• **Lamivudine** (Epivir, 3TC), Glaxo Wellcome
  Approved for combination use with AZT as a treatment option for HIV infection in adults and pediatrics patients greater than or equal to 3 months old.

• **Lamivudine/Zidovudine** (Combivir), Glaxo Wellcome
  Approved for the treatment of HIV infection in adults and adolescents greater than or equal to 12 years old.

• **Leukocyte interleukin injection** (Multkine), Cel SCI
  Currently in Phase I clinical trials for HIV.

• **Megestrol acetate** (Megace, Ovarian), Mead Johnson Laboratories
  Approved for the treatment of anorexia, cachexia, or unexplained, significant weight loss in patients with AIDS.

• **Mycophenolate mofetil** (CellCept), Hoffmann La Rouche Inc.
  Currently in Phase I clinical trials for the treatment of HIV.

• **Nelfinavir mesylate** (NFV, Viracept), Agouron Pharmaceuticals
  Approved for the treatment of HIV infection when antiretroviral therapy is warranted in adults and pediatrics greater than or equal to 2 years old. Accelerated Approval

• **Nevirapine** (Viramune, BI-RG-587), Boehringer Ingelheim Pharmaceuticals, Inc.
  Approved for use in combination with nucleoside analogues for the treatment of HIV-infected adults experiencing clinical and/or immunologic deterioration. Accelerated Approval

• **Paclitaxel** (Taxol), Bristol Myers-Squibb Pharmaceutical Research Institute
  Approved for the second-line treatment of AIDS-related Kaposi's sarcoma.
• Pentamidine (aerosolized) (NebuPent), Fujisawa
  Approved for the prevention of Pneumocystis carinii pneumonia.

• PolyI-polyC12U (Ampligen), HemispheRX
  Currently in Phase II clinical trials for treatment of HIV.

• Procaine HCL (Anticort), Samaritan Pharmaceuticals
  Phase II clinical trials for treatment of HIV completed.

• Racivir (racemic FTC), Pharmasset
  Currently in Phase II clinical trials for the treatment of HIV.

• REV 123, Novartis Pharmaceuticals
  Currently in Phase I/II clinical trials for treatment of HIV.

• Rifabutin (Ansamycin, Mycobutin), Adria Laboratories
  Approved for the prevention of Mycobacterium avium complex in patients with advanced HIV.

• Ritonavir (Norvir, ABT-538), Abbott Laboratories
  Approved for use alone or in combination with nucleoside analogues for the treatment of HIV infection. Accelerated Approval
  Approved for the use of alone or in combination with nucleoside analogues for the treatment of HIV infection in selected patients.
  Approved soft gelatin, 100 mg capsule

• S-1360 (GW810781) (integrase inhibitor), GlaxoSmithKline
  Currently in Phase II clinical trials for the treatment of HIV.

• Saquinavir mesylate (Invirase [hard gel capsule], SQV), Hoffmann-La Roche
  Approved for combination use with nucleoside analogues for the treatment of advanced HIV infection in selected patients. Accelerated Approval
  Approved soft gel capsule

• Somatropin rDNA (Serostim), Serono Laboratories
  Approved for the treatment of AIDS-wasting and cachexia. Accelerated Approval

• Stavudine (d4T, Zerit), Bristol Myers-Squibb
  Approved for the treatment of adults with advanced HIV infection who are intolerant to or deteriorating on approved therapies. Accelerated Approval and Parallel Track
  Approved for the treatment of adults with HIV infection who have undergone prolonged treatment with AZT.
Approved for the treatment of pediatrics with HIV infection who have undergone prolonged prior AZT therapy.

- **Sulfamethoxazole/Trimethoprim**, Glaxo Wellcome
  (Bactrim when combined with Trimethoprim; Septra when combined with Trimethoprim; SMX)
  Approved for the treatment of Pneumocystis carinii pneumonia (PCP)
  Approved for the prevention of PCP.

- **Tipranavir** (PNU-140690), Boehringer Ingelheim
  Currently in Phase II clinical trial for the treatment of HIV.

- **TMC125** (non-nucleoside reverse transcriptase inhibitor, Tibotec
  Currently in Phase II clinical trials for the treatment of HIV.

- **Trimethoprim/Sulfamethoxazole**, Hoffmann La-Roche
  (Bactrim when combined with Sulfamethoxazole; Septra when combined with Sulfamethoxazole; SMX)
  Approved for the treatment of Pneumocystis carinii pneumonia (PCP)
  Approved for the prevention of PCP.

- **Trimetrexate glucuronate** (with Leucovorin)(Neutrexin, TMTX), U.S. Bioscience
  Approved for the treatment of moderate to severe Pneumocystis carinii pneumonia when intolerant or refractory to TMP/SMX or when TMP/SMX is contraindicated.

- **Trizivir** (fixed-dose combination of Ziagen (abacavir/ABC), Retrovir
  (zidovudine/AZT), and Epivir (lamivudine/3TC)), Glaxo Wellcome
  Approved for the treatment of HIV in adults and adolescents. Trizivir is not recommended for treatment in adults or adolescents who weigh less than 40 kilograms because it is a fixed-dose tablet.

- **Valcyte (oral valganciclovir HCL)**, Roche Laboratories
  Approved for the treatment of cytomegalovirus (CMV) retinitis in patients with acquired immunodeficiency syndrome (AIDS).

- **Viread (tenofovir disoproxil fumarate)**, Gilead Sciences
  Approved for treatment of HIV-1 infection in combination with other antiretroviral medicines. Viread is the first nucleotide analog approved for HIV-1 treatment.
  Nucleotides are similar to nucleoside analogs, and block HIV replication in the same manner.

- **Z-100** (Ancer 20), Zeria Pharmaceutical Company
Currently in Phase I trials for the treatment of HIV.

- **Zalcitabine** (ddC, Dideoxycytidine, HIVID), Hoffmann-La Roche
  Approved for combination use with AZT for the treatment of selected patients with advanced HIV disease. This approval was based on the accelerated approval regulations.
  Approved for monotherapy treatment of advanced HIV for those aged more than 13 years who are intolerant to or have disease progression on AZT.

- **Zidovudine** (Azidothymidine, AZT, Retrovir, ZDV), Glaxo Wellcome
  Approved for the treatment of adult AIDS, or symptomatic HIV and CD4 less than or equal to 200; in syrup formulation; in intravenous dosage form; for use in early symptomatic HIV disease and in asymptomatic HIV infection where there is evidence of impaired immunity; for the treatment of pediatric HIV infection (ages 3 months to 12 years); for the prevention of perinatal transmission in HIV POSITIVE pregnant women between 14 and 34 weeks gestation and for newborns of HIV POSITIVE mothers.

A more comprehensive list of compounds that can be administered in combination and/or alternation with the composition of the present invention include (1S,4R)-4-[2-amino-6-cyclopropyl-amino)-9H-purin-9-yl]-2-cyclopentene-1-methanol succinate ("1592", a carbovir analog; GlaxoWellcome); 3TC: (−)-β-L-2',3'-dideoxy-3'-thiacytidine (GlaxoWellcome); a-APA R18893: a-nitro-anilino-phenylacetamide; A-77003; C2 symmetry-based protease inhibitor (Abbott); A-75925: C2 symmetry-based protease inhibitor (Abbott); AAP-BHAP: bis(heteroaryl)piperazine analog (Upjohn); ABT-538: C2 symmetry-based protease inhibitor (Abbott); AzddU:3'-azido-2',3'-dideoxyuridine; AZT: 3'-azido-3'-deoxythymidine (GlaxoWellcome); AZT-p-ddI: 3'-azido-3'-deoxythymidinyl-(5',5')-2',3'-dideoxyinosinic acid (Ivax); BHAP: bis(heteroaryl)piperazine; BILA 1906: N-{1S-[[3-[2S-{(1,1-dimethylethyl)amino]-carbonyl}-4R]-3-pyridinylmethyl]thio]-1-piperidinyl}-2R-hydroxy-1S-(phenylmethyl)-propyl]amino]-carbonyl]-2-methylpropyl}2-quinolinecarboxamide (Bio Mega/Boehringer-Ingelheim); BILA 2185: N-(1,1-dimethylethyl)-1-[2S-[2-2,6-dimethyl-phenoxy]-1-oxoethyl]amino]-2R-hydroxy-4-phenylbutyl][4R-pyridinylthio]-2-piperidine-carboxamide (BioMega/Boehringer-Ingelheim); BM-51.0836: thiazolo-isodindolone derivative; BMS 186,318: aminodiol derivative HIV-1 protease inhibitor (Bristol-Myers-Squibb); d4API: 9-[2,5-dihydro-5-(phosphonometoxy)-2-
furanyladenine (Gilead); d4C: 2',3'-didehydro-2',3'-dideoxyctydine; d4T: 2',3'-didehydro-3'-deoxythymidine (Bristol-Myers-Squibb); ddC; 2',3'-dideoxyctydine (Roche); ddI: 2',3'-dideoxyinosine (Bristol-Myers-Squibb); DMP-266: a 1,4-dihydro-2H-3, 1-benzoxazin-2-one; DMP-450: [(4R-(4-a,5-a,6-b,7-b)]-hexahydro-5,6-bis(hydroxy)-1,3-bis(3-amino)phenylmethyl)-4,7-bis(phenylmethyl)-2H-1,3-diazepin-2-one]-bismesylate (Avid); EBU-dM:5-ethyl-1-ethoxymethyl-6-(3,5-dimethylbenzyl)-uracil; E-BU: 5-ethyl-1-ethoxymethyl-6-benzyluracil; DS: dextran sulfate; E-EPSeU:1-(ethoxymethyl)-(6-phenylselenyl)-5-ethyluracil; E-EPU: 1-(ethoxymethyl)-(6-phenyl-thio)-5-ethyluracil; FTC:β-2',3'-dideoxy-5-fluoro-3'-thiacytidine (Triangle); HBY097:4-4-isopropoxy-carbonyl-6-methoxy-3-(methylthio-methyl)-3,4-dihydroquinazolin-2(1H)-thione; HEPT: 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine; HIV-1 human immunodeficiency virus type 1; JM2763: 1,1'-(1,3-propanediyl)-bis-1,4,8,11-tetraaza-cyclooctadecane (Johnson Matthey); JM3100:1,1'-[1,4-phenylenebis(methylene)]-bis-1,4,8,11-tetraaza-cyclooctadecane (Johnson Matthey); KNI-272: (2S,3S)-3-amino-2-hydroxy-4-phenylbutyric acid-containing tripeptide; L-697,593;5-ethyl-6-methyl-3-(2-phthalamido-ethyl)pyridin-2(1H)-one; L-735,524: hydroxyamino-pentamide HIV-1 protease inhibitor (Merck); L-697,661; 3-[[(-4,7-dichloro-1,3-benoxazol-2-yl)methyl]amine]-5-ethyl-6-methyl-pyridin-2(1H)-one; L-FDDC: (-)β-L-5-fluoro-2',3'-dideoxyctydine; L-FDOC:(-)β-L-5-fluoro-dioxolane cytosine; MKC442:6-benzyl-1-ethoxymethyl-5-isopropyluracil (I-EBU; Triangle/Mitsubishi); Nevirapine:11-cyclo-propyl-5,11-dihydro-4-methyl-6H-dipyridol[3,2-b:2',3'-e]-diazepin-6-one (Boehringer-Ingelheim); NSC648400:1-benzyloxymethyl-5-ethyl-6-(alpha-pyriddylthio)uracil (E-BPTU); P9941: [2-pyridylacetyl-IlePheAla-y(CHOH)2 (Dupont Merck); PFA: phosphonoformate (foscarnet; Astra); PMEA: 9-(2-phosphonylmethoxyethyl)adename (Gilead); PMPA: (R)-9-(2-phosphonylmethoxypropyl)adename (Gilead); Ro 31-8959: hydroxyethylamine derivative HIV-1 protease inhibitor (Roche); RPI-312: peptidyl protease inhibitor, 1-[(3S)-3-(α,β-benzyloxycarbonyl)-l-asparaginyl]-amino-2-hydroxy-4-phenylbutryl]-n-tert-butyl-l-proline amide; 2720: 6-chloro-3,3-dimethyl-4-(isopropenylxycarbonyl)-3,4-dihydro-quinolxalin-2(1H)-thione; SC-52151: hydroxyethylurea isostere protease inhibitor (Searle); SC-55389A: hydroxyethyl-urea isostere protease inhibitor (Searle); TIBO R82150: (+)-(5S)-4,5,6,7-tetrahydro-5-methyl-6-(3-methyl-2-butenyl)-imidazo-[4,5,1-jk]-[1,4]-benzodiazepin-2(1H)-thione (Janssen); TIBO 82913: (+)-(5S)-4,5,6,7-tetrahydro-9-chloro-5-methyl-6-(3-methyl-2-butenyl)imidazo[4,5,1jk]-[1,4]benzo-diazepin-2(1H)-thione (Janssen); TSAO-m3T:2',5'-bis-O-(tertbutyldimethylsilyl)-3'-spiro-5'-(4'-amino-1',2'-oxathiole-2',2'-dioxide)]-9-D-pento-
furanosyl-N3-methyl-thymine; U90152: 1-[3-[(1-methylethyl)-amino]-2-pyridinyl]-4-[[5-
[(methylsulphonyl)-amino]-1H-indol-2-y1]carbonyl]piperazine; UC:thiocarboxanilide
derivatives (Uniroyal); UC-781: N-[4-chloro-3-(3-methyl-2-butyloxy)phenyl]-2-methyl-3-
furan-carbothio-amide; UC-82: N-[4-chloro-3-(3-methyl-2-butyloxy)phenyl]-2-methyl-3-
 thiophene-carbothioamide; VB 11,328: hydroxyethyl-sulphonamide protease inhibitor
(Vertex); VX-478:hydroxyethylsulphonamide protease inhibitor (Vertex); XM 323: cyclic
urea protease inhibitor (Dupont Merck).
Adjuvants that Can Be Used in Combination and/or Alternation With the Composition of the Present Invention

An adjuvant can be used in combination with the cocktail to potentially increase the therapeutic benefit to the patient. In one embodiment, the adjuvant increases the immune response to natural antibodies in the HIV-patient. In another embodiment, the adjuvant may enhance the activity of a component of the cocktail.

In addition, if desired, the vaccine may contain auxiliary substances such as wetting or emulsifying agents, pH buffering agents or adjuvants that enhance the effectiveness of the vaccines. As non-limiting examples, the heterologous plasma cocktail can be administered in combination or alternation with any of the following known adjuvants.

Adjumer (PCPP salt; polyphosphazene; polydi(carboxylatophenoxy)phosphazene) which may be administered in the soluble form as an adjuvant for parenteral formulations or in the crosslinked form as a microsphere hydrogel for mucosal formulations. It induces a sustained antibody response after a single parenteral immunization and these antibody responses include antigen specific IgG1 and IgG2a with sustained IgG and IgA responses also induced after mucosal immunization. Algal Glucan (also known as β-glucan or glucan) is administered with antigen for enhancement of both humoral and cell-mediated immunity.

β-Glucans exert their immunostimulatory activities by binding to specific β-glucan receptors on macrophages. This ligand-receptor interaction results in macrophage activation and, in certain formulations, promotes antigen targeting. Algammulin (gamma inulin/alum composite adjuvant) is used in formulations as a primary adjuvant and stimulates immune responses by causing ligation of leukocyte-surface complement receptors (CR) via known biochemical mechanisms, thus placing the antigen close to activated leukocytes. Addition of Algammulin is known to enhance both humoral and cell-mediated immunity from either Th1 or Th2 pathways, depending on the weight ratio of inulin to Alhydrogel. Avridine (N,N-diocitadecyl-N',N'-bis(2-hydroxyethyl) propanediamine; CP20,961) may be incorporated into a liposomal preparation; into aqueous suspensions from alcoholic solution; in Intralipid, an aqueous soybean oil emulsion vehicle; other vegetable and mineral oil vehicles; in Tween 80 dispersions in saline; in saline suspension with alum-precipitated antigen. It has been shown to cause humoral and cellular immunity, proliferation of B and T lymphocytes, protective immunity, activation of macrophages, induction of interferon, enhancement of mucosal
immunity when administered orally/enterically with antigen, adjuvanticity with a variety of antigens, induction of IgG2a and IgG2b isotypes. BAY R1005 (N-(2-Deoxy-2-L-leucylamino-β-D-glucopyranosyl)-N-octadecyldecanoynamide hydroacetate) can be used as a primary adjuvant. BAY R1005 in combination with purified virus vaccines or subunit vaccines led to increased protection of virus-challenged mice. The increase in antibody synthesis induced by BAY R1005 is specifically dependent on the antigen and it acts on the proliferation of B lymphocytes as a second signal which has no effect until the antigen acts as a first signal. BAY R 1005 is capable of activating B lymphocytes without the helper function of T lymphocytes. In mice parenteral immunization with recombinant urease mixed with BAY R1005 induced strong Th1 and Th2 responses and thereby elicited better protection against Helicobacter pylori infection than adjuvants which induced a prominent Th2 type response only (Guy, B., et al., 1998. Systemic immunization with urease protects mice against Helicobacter pylori infection. Vaccine 16:850-856.) Calcitriol (1α, 25-dihydroxyvitamin D3; 1,25-di(OH)2D3; 1,25-DHCC; 1α, 25-dihydroxycholecalciferol; 9,10-seco(5Z,7E)-5,7,10(19)-cholestatriene- 1α,3β,25-triol) has been shown to promote the induction of mucosal and systemic immunity when incorporated into vaccine formulations. Calcium Phosphate Gel has been used as adjuvant in vaccine formulations against diphtheria, tetanus, pertussis and poliomyelitis. It adsorbs soluble antigens and presents them in a particulate form to the immune system and contains no components that are not natural constituents of the body and is very well tolerated. Cholera toxin B subunit (CTB, also known as CTB subunit) augments humoral responses by acting as an efficient carrier/delivery system and is completely non-toxic and has been used extensively in humans without negative side-effects. Cholera holotoxin (CT) has been shown to augment both humoral and cell-mediated immunity, including CTL responses, and thereby enhances MHC class I and II restricted responses. CT exerts immunomodulating effects on T cells, B cells as well as antigen-presenting cells (APC). Cholera toxin A1-subunit-Protein A D-fragment fusion protein (CTA1-DD gene fusion protein) has proven equivalently potent as an adjuvant to the intact cholera holotoxin (CT) for humoral and cell-mediated immunity. CTA1-DD is targeted to B lymphocytes, both memory and naïve cells and acts as a powerful systemic and mucosal adjuvant.

Block Copolymer P1205 (CRL1005) acts as both an adjuvant and stabilizer and forms microparticulate structures that can bind a variety of antigens via a combination of hydrophobic interactions and surface charge. Cytokine-containing Dehydration Rehydration
Vesicles (Cytokine-containing Liposomes) induces both cellular and humoral immunity. Dimethyl dioctadecylammonium bromide; dimethyldistearylammonium bromide (DDA - CAS Registry Number 3700-67-2) is known for stimulation immune responses against various antigens and especially delayed type hypersensitivity. DHEA (Dehydroepiandrosterone; 5-androsten-3β-ol-17-one; dehydroisoandrosterone; androstenolone; prasterone; transdehydroandrostosterone; DHA) can be directly incorporated into vaccine formulations and will enhance antibody formation. DHEA can be administered systemically at the time of vaccination, or can be directly incorporated into the vaccine formulation. DMPC (Dimyristoyl phosphatidylcholine; sn-3-phosphatidyl choline-1, 2-dimyristoyl; 1, 2-dimyristoyl-sn-3-phosphatidyl choline; (CAS Registry Number 18194-24-6)) and DMPG (Dimyristoyl phosphatidylglycerol; sn-3-phosphatidyl glycerol-1, 2-dimyristoyl, sodium salt (CAS Registry Number 67232-80-8); 1, 2-dimyristoyl-sn-3-phosphatidyl glycerol) are used in the manufacture of pharmaceutical grade liposomes, typically in combination with DMPG and/or cholesterol and are also used in adjuvant systems for vaccine formulations. DOC/Alum Complex (Deoxycholic Acid Sodium Salt; DOC/Al(OH)₃/ mineral carrier complex) is a complex used as adjuvant formulation and is known to enhance the immune response to membrane proteins. Freund's Complete Adjuvant is a mixture of mineral oil (Marco 52) and emulsifier (Arlacel A [mannide monooleate]) as an emulsion of 85% mineral oil and 15% emulsifier with heat-killed antigen. Gamma Inulin is a highly specific activator of the alternative pathway of complement in vitro and in vivo included in adjuvant formulations as a primary adjuvant and also as the immune stimulant when combined as composite particles with alum in the adjuvant Algammulin. It is expected that it stimulates immune responses by causing ligation of leukocyte-surface complement receptors (CR) via known biochemical mechanisms. Addition of gamma inulin is known to enhance both humoral and cell-mediated immunity from both Th1 and Th2 pathways. Gamma inulin also has an antitumor action and an effect on natural immunity. Gerbu Adjuvant, an adjuvant based on GMDP with DDA and Zinc-L-proline have been shown to complex as synergists. GM-CSF (Granulocyte-macrophage colony stimulating factor; Sargramostim (yeast-derived rh-GM-CSF)) is a glycoprotein of 127 amino acids and recombinant human GM-CSF is produced in yeast and it differs from the natural human GM-CSF by substitution of Leu for Arg at position 23. This cytokine is a growth factor that stimulates non-nal myeloid precursors, and activates mature granulocytes and macrophages.
GMDP (N-acetylglucosaminyl-(β1-4)-N-acetylmuramyl-L-alanyl-D-isoglutamine (CAS Registry Number 70280-03-4) - Semi-synthetic. Disaccharide isolated from microbial origin, dipeptide wholly synthetic. US Pat. No. 4,395,399) is known as a primary adjuvant. It has been shown to be an highly effective primary adjuvant in a range of vehicles; aqueous buffers, mineral oil, pluronic/squalane/Tween emulsions. Also effective as oral adjuvant, enhancing mucosal IgA response. Imiquimod (1-(2-methylpropyl)-IH-imidazo[4,5-c]quinolin-4-amine; R-837; S26308) can be included in adjuvant formulations as a primary adjuvant component and is known to induce both humoral and cell-mediated immunity via induction of cytokines from monocytes and macrophages. ImmTher™ (N-acetylglucosaminyl-N-acetylmuramyl-L-Ala-D-isoGlu-L-Ala-glycerol dipalmitate; DTP-GDP) is a potent macrophage activator which induces high levels of TNF, IL-1, and IL-6 both in vitro and in vivo (US Patent 4,950,645). Immunoliposomes prepared from Dehydration-Rehydration Vesicles (DRV) (Immunoliposomes Containing Antibodies to Costimulatory Molecules) are composed of phosphotidylcholine/cholesterol/biotinylate d-phosphatidylethanolamine (PC/CH/PEB) in a molar ration of 5:5:1. Antigen is added to the water suspension of DRV followed by repeated vortexing and lyophylization of the liposome suspension. Interferon-γ (Actimmune® (rhIFN-gamma, Genentech, Inc.); immune interferon; IFN-γ; gamma-interferon) has demonstrated higher and earlier neutralizing antibody titers, an increase in duration of neutralizing antibody titers, an increase in MHC class II expression on antigen presenting cells, increase in Helper T cell levels, and an improved DTH response. The IFN-gamma is preferably given at the same site and at the same time (within 6 hrs) as the antigen. Interleukin-1β (IL-10; IL-1; human Interleukin 1β mature polypeptide 117-259) is known as a primary adjuvant and is active by oral, intravenous, intraperitoneal and subcutaneous routes. It increases both T-dependent and T-independent responses to different types of antigens and can be active in both primary and secondary responses. Interleukin-2-(IL-2; T-cell growth factor; aldesleukin (des-alanyl-1, serine-125 human interleukin 2); Proleukin®; Teceleukin®) is used as a primary adjuvant, co-emulsified with antigens and lipids, with polyethylene glycol modified long acting form (PEG IL-2), or liposome encapsulated sustained release dosage form. IL-2 supports the growth and proliferation of antigen-activated T lymphocytes and plays a central role in the cascade of cellular events involved in the immune response. Proliferating T-cells also produce a variety of other lymphokines which may modulate other arms of the immune system and in view of these direct and indirect actions of IL-2 on the immune response, IL-2
functions as an adjuvant to vaccination by increasing the specific and durable response to vaccine immunogens. Low doses may give up to 25-fold increase in adjuvant effect, with inhibition of adjuvant effect at high doses. May induce cellular immunity when given systemically, and IgA when administered at a mucosal surface. Interleukin-7 (IL-7) has been shown to enhance antibody production as a primary adjuvant in liposome formulated sustained release form (Bui, et al. "Effect of MTP-PE liposomes and interleukin-7 on induction of antibody and cell-mediated immune responses to a recombinant HIV-envelope protein", J Acquired Immune Deficiency Syndrome, 1994 Aug;7(8):799-806.) and has also been used co-emulsified with antigen and lipids. Interleukin-12 (IL-12; natural killer cell stimulatory factor (NKSF); cytotoxic lymphocyte maturation factor (CLMF)) is used as a primary adjuvant component to enhance Th1-dependent cell-mediated immune responses including cytolytic T-lymphocyte responses.

Immune stimulating complexes (ISCOMs\textsuperscript{(s)}) are a complex composed of typically Quillaja saponins, cholesterol, phospholipid, and antigen in phosphate-buffered saline (PBS). They are antigen-presenting structures that have been shown to generate long-lasting biologically functional antibody response. ISCOMs have demonstrated a protective immunity and a functional cell-mediated immune response, including Class I restricted CTLs have been reported in several systems. They have generally been administered subcutaneously or intramuscularly but non-parenteral administrations (intranasal and oral) have also proven to be effective. Liposomes (L) containing protein or Th-cell and/or B-cell peptides, or microbes with or without co-entrapped interleukin-2, BisHOP or DOTMA A, L (Antigen)]; B, [L (IL-2 or DOTMA or BisHOP + Antigen)]; C, [L (Antigen)-mannose]; D, [L (Th-cell and B-cell epitopes)]; E, [L (microbes)] act as carrier of Th-cell peptide antigen which provides help for co-entrapped B-cell antigen to overcome genetic restriction and induce immunological memory. They may also act as carriers of attenuated or live microbial vaccines to deliver microbes and co-entrapped soluble antigens or cytokines simultaneously to antigen-presenting cells or to protect entrapped vaccines from interaction with maternal antibodies or antibodies to vaccine impurities in preimmunized subjects. Loxoribine (7-allyl-8-oxoguanosine) is known as a primary adjuvant for antibody responses to a wide variety of antigen types in a variety of species. It augments CTL-mediated, NK cell-mediated, macrophage mediated, and LAK cell-mediated cytotoxicity, induces IFN(α/β/γ, TNΦα, TNΦβ, IL-1α, IL-6 and up regulates humoral immune responses in immunodeficiency. LT-OA or LT Oral Adjuvant induces both
mucosal and systemic immunity (both humoral [including IgA and IgG2, isotypes] and cell-mediated) to killed microorganisms or peptide antigens mixed with it in neutral non-phosphate buffered saline, with/without sodium bicarbonate. MF59 (Squalene/water emulsion - composition: 43 mg/mL squalene, 2.5 mg/mL polyoxyethylene sorbitan monooleate (Polysorbate 80), 2.4 mg/mL sorbitan trioleate (Span 85)) in combination with a variety of subunit antigens results in elevated humoral response, increase T cell proliferation and presence of cytotoxic lymphocytes. MONTANIDE ISA 51 (Purified IFA; Incomplete Freund's adjuvant) addition induces humoral and cell-mediated immunity with various antigens. MONTANIDE ISA 720 (metabolizable oil adjuvant) induces humoral and cell-mediated immunity with various antigens. MPL* (3-Q-desacyl-4'-monophosphoryl lipid A; 3D-MLA) is used as a primary adjuvant in adjuvant formulations. Its activity is manifested either alone in aqueous solution with antigen, or in combination with particulate vehicles (e.g., oil-in-water emulsions) and its activity may be enhanced by use of vehicle that enforces close association with antigen. MTP-PE (N-acetyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1,2-dipalmitoyl-sn-glycero-3-(hydroxy-phosphoryloxy)) ethylamide, monosodium salt) and alternately MTP-PE liposomes are optionally a part of MF59 and are known as immunomodulators. The addition of MTP-PE to the MF59-based HIV vaccine in HIV seropositive individuals resulted in a marked increase in HIV antigen lymphocyte proliferation. Murametide (Nac-Mur-L-Ala-D-Gln-OCH3) induces granulocytosis and enhances the humoral response. Murametide displays the same profile of adjuvant activity as MDP and has been chosen for development because of its favorable therapeutic ratio. When administered in 50% water-in-oil emulsion, it mimics the activity of Freund's complete adjuvant without its side effects (US Patent No. 4,693,998.) Murapalmitine (Nac-Mur-L-Thr-D-isogln-sn-glycero1 dipalmitoyl) is administered in water-in-oil emulsion as an adjuvant of humoral and cell-mediated responses. D-Murapalmitine (Nac-Mur-D-Ala-D-isogln-sn-glycerol dipalmitoyl) is a strong adjuvant of humoral and cell-mediated immunity when administered in a 50% mineral oil emulsion. NAGO is a mixture of the two enzymes-neuraminidase and galactose oxidase Ag 1:5 ratio in units of activity. It generates cell surface Schiff base-forming aldehydes on antigen presenting cells and Th-cells, thereby amplifying physiologic Schiff base formation that occurs between cell-surface ligands as an essential element in APC:T-cell inductive interaction. It is a potent non-inflammatory adjuvant with viral, bacterial and protozoal subunit vaccines, and is especially effective in the generation of cytotoxic T-cells.
Non-Ionic Surfactant Vesicles (NISV) induces both a humoral and cell-mediated immune response and preferentially stimulates the Th1 sub-population of T-helper cells. It is known to be effective with antigens within a broad size range, from short peptides to particulates, and has extremely low toxicity. Pleuran (β-glucan; glucan) has shown in experimental studies that rabbits as well as mice immunized once by coadministration of viral antigens and 60 μg of Pleuran produced at least 20-fold higher antibody titers than control animals injected with the immunogen alone (Chihara, G. et al., 1989, Lentinan as a host defense potentiator (HDP), *Int. J. Immunother.* 4:145-154.) PLGA, PGA, and PLA (Homo-and co-polymers of lactic and glycolic acid; Lactide/glycolide polymers; poly-lactic-co-glycolide) used in vaccine delivery have demonstrated an ability to control the release of antigen after administration, thereby eliminating or reducing the need for boost immunizations. Antigens incorporated in PLGA microspheres have exhibited enhanced and prolonged antibody activity responses compared to equivalent doses of free antigen. Pluronic L121 (poloxamer 401) enhances the presentation of antigen to cells of the immune system. PMMA (polymethyl methacrylate) is known as a primary adjuvant for all types of antigens. PODDS™ (proteinoid microspheres) serves as a vehicle for oral immunization, protecting the antigen and allowing for co-encapsulation of adjuvants with antigens in microspheres. Poly rA:Poly rU (a double helix comprised of polyadenylic acid and polyuridylic acid) is known as an adjuvant to humoral and cell-mediated immunity when given with antigen; it increases non-specific immunity to microorganisms. Polysorbate 80 may be used in emulsion vaccine formulations including MF59, SAF-1 and Antigen Formulation. Protein cochleates act as both carriers and adjuvants, providing multivalent presentation of antigens to the immune system, with maintenance of native conformation and biological activity and providing protection of antigens from degradation following oral delivery. They stimulate strong mucosal and systemic antibody, proliferative and cytotoxic responses to associated antigens. QS-21 (Stimulon™ QS-21 Adjuvant) can be used in vaccine formulations as a primary adjuvant component for enhancement of both humoral and cell-mediated immunity. Quil-A (Quil-A saponin, *Quillaja* saponin) is used in veterinary vaccines and for production of ISCOMs. Rehydragel HPA (High Protein Adsorbency Aluminum Hydroxide Gel; alum) and Rehydragel LV (low viscosity aluminum hydroxide gel; alum) are primary adjuvants in parenteral vaccine formulations and aluminum compounds (aluminum hydroxide, aluminum phosphate, alum) are currently the only vaccine adjuvants used in US-licensed vaccines. The use of aluminum adjuvants are accompanied by stimulation of IL-4 and stimulation of the T-
helper-2 subsets in mice, with enhanced IgG1 and IgE production. S-28463 (4-Amino-otic-, 5
dimethyl-2-ethoxymethyl-1H-imidazo[4,5-c]quinoline-1-ethanol) induces both humoral and cell-mediated immunity via induction of cytokines from monocytes and macrophages. Experimental results indicate S-28463 is about 100-fold more potent than imiquimod in antiviral models and in cytokine induction from monocytes and macrophages. Syntex Adjuvant Formulation (SAF, SAF-1, SAF-m) causes antigens to arrange on the surface of the emulsion droplets partly because of their amphipathic nature, and partly because of hydrogen bonding with poloxamer 401. The emulsion droplets also activate complement, as demonstrated by consumption of C3 and production of C3b; the latter, on the surface of droplets, targets them to antigen-presenting cells (follicular dendritic cells and interdigitating cells) in lymph nodes of the drainage chain and possibly in more distant lymphoid tissues. In this way the emulsion facilitates the presentation of antigens to responding lymphocytes (threonyl-MDP monograph). Sclavo peptide (IL-1β 163-171 peptide) enhances immune response to T-dependent and T-independent antigens. It is known as a primary adjuvant and may be administered i.p, i.v., s.c. or p.o and it is active either when administered separately from antigen, or admixed with antigen, or physically linked to antigen. Sendai Proteoliposomes, Sendai-containing Lipid Matrices (Sendai glycoprotein-containing vesicles; fusogenic proteoliposomes; FPLs; Sendai lipid matrix-based vaccines) are potent immunogens and have the ability to stimulate strong T helper and CD8+ cytotoxic T cell responses (CTL) to lipid bilayer-integrated glycoproteins as well as encapsulated peptides, proteins and whole formalin-fixed viruses. These vesicles also act as effective delivery vehicles for drugs and proteins.

Span 85 (Arlacel 85, sorbitan trioleate) is used as an emulsification agent in MF59 adjuvant formulation. Specol (Marcol 52 (mineral oil, paraffins, and cycloparaffins, chain length 13-22 C atoms) Span 85 (emulsifier, sorbitan trioleate) Tween 85 (emulsifier, polyoxyethylene-20-trioleate)) all are individually FDA approved for veterinary use and they function as a depot (slow release of antigen) and a polyclonal activator (independent of presence of antigen) for cells of the immune system (cytokine release). Squalane (Spinacane;Robane®;2,6,10,15,19,23-hexamethyltetraicosane) is a component of Antigen Formulation (AF) and Syntex Adjuvant Formulation (SAF), and constitutes the oil component of the emulsion. Stearyl Tyrosine (octadecyl tyrosine hydrochloride) has adjuvanticity similar to aluminum hydroxide with bacterial vaccines; superior to aluminum hydroxide with viral vaccines. Theramide™ (N-acetylglucosaminy1-N-acetylinuramyl-L-
Ala-D-isoGlu-L-Ala-dipalmitoxygen propylamide (DTP-DPP)) is a potent macrophage activator and adjuvant. It induces IL-6, IL-12, TNF, IFN-γ, and relatively lesser quantities of IL-10. The compound preferentially induces cellular immunity. Threonyl-MDP (Termurtide™; [thr]-MDP; N-acetyl muramyl-L-threonyl-D-isoglutamine) induces the production of a cascade of cytokines, including IL-1a, IL-1β and IL-6. Responding lymphocytes release IL-2 and IFN-γ and the latter increases the production of antibodies of certain isotypes, including IgG2a. This isotype, and the homologous IgG1 in primates, interacts with high affinity Fcγ receptors, so that the antibodies can function efficiently in opsonizing viruses and other infectious agents for uptake by phagocytic cells. Ty Particles or Ty Virus-Like Particles present antigen in a polyvalent, particulate form. Cytotoxic T-lymphocytes are induced in the absence of any other adjuvant formulations. Walter Reed Liposomes (Liposomes containing lipid A adsorbed to aluminum hydroxide, [L(Lipid A + Antigen) + Alum]) have been shown to induce both humoral and cell-mediated immunity. Liposomes containing lipid A provide a very potent adjuvant activity. Adsorption of liposomes containing lipid A to aluminum hydroxide gel contributes additional strong adjuvant activity.

**Pharmaceutical Compositions**

Humans suffering from HIV can be treated by administering to the patient an effective amount of the defined heterologous plasma or serum composition. In one embodiment of the present invention, the heterologous plasma or serum composition is delivered alone. In another embodiment of the present invention, the heterologous plasma or serum composition can be formulated as a pharmaceutical composition. Pharmaceutical compositions of the invention may also include suitable carriers, excipients, diluents, vehicles, depending upon the intended use. A general review is provided in “Remington’s Pharmaceutical Sciences” by E.W. Martin, 16th edition, Osol, A. Ed. (1980).

The cocktail can be formulated in any manner that achieves the desired results and maintains the activity of the material. Treatment can include, for example, administering the compositions of the present invention parenterally (i.e., intravenous, intraarterial, intraperitoneal, intramuscular, subcutaneous intracorporeal, intrasternal, intraventricular, intrathecal, intra-articular, intra-synovial, intrahepatic, intralesional and intracranial injection or infusion techniques) intranasally, intravaginally, orally, rectally, topically (i.e., by
powders, ointments, drops or transdermal patch) or by any other effective method of administration.

The composition can be formulated for parenteral administration, which involves the administration of liquid through some other route than the digestive track. Pharmaceutical compositions for parenteral injection comprise pharmaceutically acceptable sterile aqueous or nonaqueous (i.e., oily) solutions, dispersions, suspensions or emulsions as well as sterile powders (i.e., dry lyophilized powder or water free concentrates) for reconstitution into sterile injectable solutions or dispersions just prior to use. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents or vehicles include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol and the like), aqueous dextrose and related sugar solutions, Ringer’s solution oils (including those of animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like) and injectable organic esters such as ethyl oleate. The pharmaceutical carrier should be compatible with the intended use of the composition. Forms for parenteral administration can also contain suspending, stabilizing and/or dispersing agents. The formulation may include substances that enhance isotonicity and chemical stability, including buffers (i.e., phosphate, citrate, succinate, acetic acids and other organic acids and their salts); antioxidants (e.g., sodium bisulfite, sodium sulfite, or ascorbic acid, either alone or combined), hydrophilic polymers, amino acids, monosaccharides, disaccharides and other carbohydrates (i.e., cellulose); chelating agents (i.e., EDTA); sugar alcohols (i.e., manitol); counterions (i.e., sodium); and/or nonionic surfactants (PEG). In addition, parenteral solutions can contain preservatives, such as benzalkonium chloride, methyl- or propylparaben, and chlorobutanol.

The compositions may be presented in unit-dose or multidose sealed containers, for example, ampoules, vials, disposable syringes made from glass or plastic. When the composition is provided as a powder (i.e., lyophilized powder, generally including an inert diluent or edible carrier) for reconstitution with liquid prior to use, the ingredients are supplied separately. For example, the sterile powder (i.e., lyophilized powder) is provided in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to injection.
In a preferred embodiment, the plasma cocktail composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration or injection to human beings. Intravenous techniques are the principal means of parenterally delivering drugs to humans. The delivery vehicles used in connection with such techniques are physiologically compatible sterile aqueous solutions. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Other preferred carriers for injectable solutions include saline solutions and aqueous dextrose and glycerol solutions. Injectable forms may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection.

The plasma cocktail can be formulated as neutral or salt forms. Pharmacologically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, taratic acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

In one embodiment of the present invention, the composition is delivered by bolus injection. In another embodiment, the plasma cocktail can be administered continuously. Continuous administration may be performed according to any of the methods and techniques known to those skilled in the art, including continuous injection or infusion using a surgically implantable pump, such as an Arrow Model 3000 (Arrow International, Walpole, Mass.) or MiniMed 2001 (MiniMed Technologies, Sylmar, Calif.); injection or infusion using an externally worn pump, such as the MiniMed 504S (MiniMed Technologies) or the H-Tron V100 (Disetronic Medical Systems, Minnetonka, Minn.); or diffusion from a biologically-derived bio-resorbable hydrogel, such as a chitosan hydrogel or N,O-carboxymethyl chitosan (NOC-chitosan) hydrogel, alone or in combination with a charged or uncharged polymeric agent to control hydrogel porosity and/or hydrogel stability and/or kinetics of protein release, such as polyllysine or polypropylene glycol; or diffusion from a synthetically-derived bio-resorbable hydrogel, such as polypropylene glycol, alone or in combination with a charged or uncharged polymeric agent to control hydrogel porosity and/or hydrogel stability and/or kinetics of protein release, such as polyllysine.

A preferred mode of administration of the active compound is through subcutaneous injection, which can optionally include an inert diluent or carrier. Preferred carriers are water, physiological saline, phosphate buffered saline (PBS), dextrose solution, or Ringer's Solution.
For oral administration, the pharmaceutical compositions can take the form of, for example, solids (i.e., tablets, pills, granules) liquids, or semi-solids (i.e., soft gelatin capsules). Forms for oral administration can be prepared by conventional means and pharmaceutically acceptable excipients or compounds similar in nature: binding agents (e.g., pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose, microcrystalline cellulose, gum tragacanth, syrup, accacia, sorbitol, mucilage of starch or gelatin); fillers (e.g., lactose, sugar, microcrystalline cellulose or calcium hydrogen phosphate, maize-starch, or sorbitol.,); lubricants (e.g., magnesium stearate, talc, Sterotes, stearic acid, talc, polyethylene glycol or silica); disintegrants (e.g., alginic acid, Primogel, or starch including potato starch, corn starch or sodium starch glycolate); wetting agents (e.g., sodium lauryl sulfate); sweetening agents (e.g., sucrose or saccharin) or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring) . Other non-limiting examples of suitable pharmaceutical excipients include glucose, malt, rice, flower, chalk, silica gel, sodium stearate, glycerol monostearate, sodium chloride, dried skim milk, glycerol, water, ethanol and the like. The tablets can be coated by methods well known in the art. When the dosage unit form is a capsule, it can contain, in addition to material of the above type, a liquid carrier such as a fatty oil. In addition, dosage unit forms can contain various other materials which modify the physical form of the dosage unit, for example, coatings of sugar, shellac, or other enteric agents.

Liquid preparations for oral administration can take the form of, for examples, solutions, suspensions (i.e., aqueous or oily), emulsions, syrups or elixirs, or they can be presented as a dry product for reconstitution with water or other suitable vehicle prior to use. Such liquid preparations can be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivates, hydrogenated edible fats glucose/sugar syrup, gelatin, aluminum stearate gel); emulsifying agents (e.g., lecithin, sorbitan mono-oleate or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (e.g., methyl or proply-p-hydroxybenzoates or sorbic acids). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate.

If administered by nasal aerosol or by inhalation, the heterologous plasma cocktail is prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable
preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other solubilizing or dispersing agents known in the art.

If administered by nasal aerosol or inhalation, the heterologous plasma cocktail is prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other solubilizing or dispersing agents known in the art.

If rectally or vaginally administered in the form of suppositories, the heterologous plasma cocktail may be prepared by mixing the drug with a suitable non-initiating excipient, for example cocoa butter, synthetic glyceride esters of polyethylene glycols, which are solid at ordinary temperatures, but liquefy and/or dissolve in the rectal cavity to release the drug. Suitable excipients for suppositories are e.g. natural or hardened oils, waxes, fats, semi-liquid or liquid polyols etc.

The heterologous plasma or serum cocktail can also be mixed with other active materials that do not impair the desired action, or with materials that supplement the desired action (i.e., to treat or prevention HIV infection/AIDS). In one embodiment, the composition can be administered in combination (i.e., co-administered or sequentially administered) with anti-viral compounds (i.e., nucleotide or non-nucleoside antiviral agents, protease inhibitors, integrase inhibitors, etc) as discussed in greater detail above. Other non-limiting examples of compounds that can be administered in combination with the composition of the present invention include antibacterial agents (i.e., benzyl alcohol, methyl parabens, anti-fungals, anti-inflammatories, as discussed in more detail above. In one embodiment of the present invention, the composition is administered in combination with a vaccine to prevent infection with HIV.

The heterologous plasma cocktail is included in the pharmaceutically acceptable carrier or diluent in an amount sufficient to deliver to a patient a therapeutically effective amount. A "therapeutically effective amount" of the compound of the invention means a sufficient amount of the compound to treat or prevent HIV infection. In one embodiment of the present invention, a therapeutically effective amount of the composition would be the amount required to lower detectable viral load at a reasonable benefit/risk ratio (i.e., benefit to side effects) applicable to any therapeutic treatment. In another embodiment, a therapeutically effect amount would be the amount required to prevent and/or lower risk of
infection at a reasonable benefit/risk ratio (i.e., benefit to side effects) applicable to any preventative treatment. It will be understood that the usage of compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of the specific compound employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration; the route of administration; the rate of excretion of the specific composition employed; the duration of the treatment; drugs used in combination or coincidental with the specific composition employed and like factors well known in the medical arts. It therefore noted that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions. For example, it is well within the skill of the art to start doses of the composition at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. The total dose of the heterologous plasma or serum composition can be administered in a single dose, or divided into a number of smaller doses to be administered at varying intervals of time.

Examples

Experiments have been conducted in vitro to show that the composition of the present invention can inhibit HIV-1 viral attachment and entry into cells, as well as inhibit viral replication of clinical strains of HIV in infected cells. HIV+ patients have used the composition of the present invention to considerable success. Results are summarized below.

Example 1. Production of the Immunogen

Patients who are HIV positive and with a detectable viral load, and preferably with a viral load above 2,000, are used for blood samples because this provides a sufficient challenge to the animal’s immune system to generate a response. The blood taken from the patient is centrifuged at 32,000 rpm at room temperature using standard sterile laboratory
techniques and the resulting patient plasma/serum is frozen at -20°C. Preferably the sample is frozen no more than 24 hours, but may be left for longer.

**Example 2. Inoculation of the Animal**

The animal used in the process is first inspected by a veterinarian and evaluated for any underlying abnormalities in the animal and for any pathogens that may cause a possible zoonosis. Once the animal is found to be healthy it is then well maintained in a clean environment and monitored by a veterinarian on a regular basis.

The HIV+ patient plasma sample prepared as outlined in Example 1 is allowed to thaw to room temperature and approximately 3cc of the patient plasma/serum is injected subcutaneously into the animal according to standard sterile procedures. Once the animal has been injected with the sample, the animal is carefully marked and labeled assigning it a number and indicating where the sample was taken and from whom. A three week period of time is allowed to pass prior to harvesting any of the animal’s blood.

**Example 3. Preparation of the Heterologous Plasma Cocktail**

The specimen animal inoculated as outlined in Example 2 is injected with 0.5 cc rompun (for ease of handling). After the animal has reached the appropriate level of anesthesia, the external jugular area is steriley prepped and draped. An 18 gauge one inch needle attached to a 60cc lure lock syringe is introduced into the external jugular vein and approximately 200 to 400cc total (using 4-8 lure lock syringes) is steriley extracted. The blood is stored in a container treated with anticoagulant. The blood is immediately transferred to an ice bath to keep it cool, immediately following which the blood is centrifuged with an office model centrifuge at 32,000 rpm room temperature and the resultant plasma mixture is steriley removed and passed through a 0.5 micron succion filtration device. The sterile product is then placed in an ice bath when transportation to main laboratory facilities is necessary, where the product is sterily filtered through a 0.2 micron succion filtration. It is then transferred to a non-refrigerated ultracentrifuge for twenty minutes at 90,000rpm after which the supernatant is sterily transferred to appropriately sized tubes for a refrigerated ultracentrifuge and spun at 150,000 rpm for twenty minutes. The supernatant is then passed through an anhydrous filter and then passed through a 0.1 micron succion filtration and into sterile containers where it is then placed into a -70°C freezer and kept for at least 48 hours. After the 48 hours have passed, samples of the batch are taken and cultured.
both anaerobically and aerobically for any pathogens. Once the culture gives negative results
the heterologous plasma cocktail is ready for patient use.

Example 4. Inhibition of Viral Replication in PHA-Stimulated PBMCs

Peripheral blood mononuclear cells were prepared from whole blood from uninfected
donors by Ficoll-Hypaque density centrifugation, pooled, and stimulated in vitro for 72 hours
with phytohemagglutinin (PHA) to activate cellular replication. PHA-stimulated blasts were
then washed and cultured in microtiter plates in the presence of 3.6 ng/mL interleukin-2 to
maintain cellular activation and replication, pretreated with increasing concentrations of
either VR-30 (TV), a plasma cocktail prepared as described in Example 3, or VR-30 (HV),
plasma from an unimmunized goat, and infected with one of four strains of HIV-1 subtype B
virus at a multiplicity of infection (MOI) of 0.05-0.10 infectious virions/cell. The HIV-1
viral strains tested were BR/92/014, HT/92/593, HT/92/596, and HT/92/599. Zidovudine
(AZT) was used as a positive control in these assays. The antiviral efficacy and toxicity of
VR-30 were evaluated at 6 days post-infection in parallel with the effects of AZT, by
measurement of HIV-1 reverse transcriptase activity or by staining with the tetrazolium dye
XTT, respectively.

The results of these studies are presented below in Tables 1-3 below. The positive
control AZT was active against all four strains of HIV-1 tested. The test agent VR-30 (TV)
was active against the HT/92/599 strain with an IC₅₀ at approximately a 1:100 dilution of
VR-30, and against the HT/92/596 strain with an IC₅₀ of approximately a 1:320 dilution
(Tables 1). VR-30 (HV) was active against the BR/92/014 strain at a 1:100 dilution, but
inactive against all other strains tested (Table 2). Direct comparison of results for the two
plasma preparation, VR-30(TV) and VR30(HV) establishes that serum from the immunized
goat is more active than serum from the unimmunized goat, although unimmunized goat
serum shows some activity (Table 3). The IC₅₀ for AZT in these assays ranged from
<0.0001 to 0.009 μM for the different strains, confirming the sensitivity of the assay.

Example 5. Inhibition of HIV-1 Viral Attachment in CD4+ Expressing Cells

The ability of VR-30 to inhibit the attachment of HIV-1 to CD4⁺-expressing target cells
expressing an HIV-activated reporter gene was determined using the HeLa-CD4-LTR-Δ-galactosidase, transgenic cell line. In this assay, inhibition of viral attachment and entry into
the target cells results in decreased expression of the reporter gene, which may be quantified
by chemiluminescence following staining with a specific, Gal-screen substrate. HeLa-CD4-
LTR-β-galactosidase cells were plated in microtiter wells overnight, then exposed to increasing dilutions of VR-30 or controls (medium; Chicago Sky Blue, or dextran sulfate as positive control agents) for 15 to 30 minutes. HIV-1 was added to the cultures for two hours, then the plates were washed extensively to remove unbound virus and returned to culture for an additional 48 hours. Following incubation, trans-activation of B-galactosidase was evaluated by chemiluminescence using a commercially available detection kit (Tropix) Gal-screen substrate was added and chemiluminescence was measured according to the recommendations of the manufacturer.

The results of this study are presented in Table 4 below. Briefly summarized, two different preparations of VR-30 were found to inhibit viral attachment and entry, with IC_{50} values ranging from 1:10 to 1:75 dilutions. Viral attachment and entry were also significantly inhibited by treatment with either of the two positive control compounds, Chicago Sky Blue or dextran sulfate (data not presented). Treatment of cells with VR-30 had no significant effects on cellular cytotoxicity or cell:cell fusion, and viability was always > 80% at all dilutions of VR-30, as compared to cells treated with medium control (data not presented).

**Example 6. In Vitro Virucidal Activity Against HIV-1**

In order to further clarify the mechanism of action, it was evaluated in a virucidal in vitro assay.

Infectious virus was mixed with VR-30 for two hours at 37°C, the virus was separated from the test article by ultracentrifugation, and the infectious titer of the resulting viral pellet was quantitated following infection of HeLa-CD4-LTR-β-galactosidase cells. Inactivation of HIV-1 by VR-30 would result in a decrease of β-galactosidase signal in each well.

The results from this assay demonstrated that there was no direct virucidal activity of VR-30 against HIV-1 strain IIIB. Aldrithiol was used as a positive control agent for this assay, and performed as expected.

Taken together with the in vitro attachment and replication results described in Examples 4 and 5, respectively, these data suggest that the anti-HIV activity of VR-30 may be mediated by a novel mechanism(s), which may include inhibition of attachment of HIV-1 to its target cell, thereby preventing viral entry and replication.

**Example 7: Antibody Depletion of the Composition**
In order to further identify the active component or components of the composition of the present invention, antibody depletion experiments were conducted.

In the panning method, anti-goat antibodies are attached to plastic, the VR-30 TV (the composition prepared according to Example 3) was added to allow antibody in the goat serum to bind to the anti-goat antibody. The residual antibody depleted material was removed and tested (in parallel with untreated or mock treated material). Experiments were completed to remove material and removed the IgG, the IgM or both the IgG and the IgM.

The results of the experiments are found in Table 5, below. The antibody depleted samples possessed the same activity as untreated or mock treated (PBS) samples. These results would suggest that the removal of the antibodies did not yield a corresponding reduction in the activity of the material. These results would tend to suggest that while antibody may be present and may possibly contribute to the observed effect, it is likely not responsible for the activity of the composition.

Example 8. Results of Treatment

For patients who have been exposed to HIV but have not tested positive for HIV it is recommended that the patient receives 2cc of therapy twice monthly for six months. The heterologous plasma cocktail is administered subcutaneously to the abdominal area. Patients who are HIV positive should receive 5cc subcutaneously monthly for one year. Patients who are HIV positive and have a CD4 count of greater than 400 should receive 5-10cc subcutaneously daily for three consecutive days then 5cc monthly for one year depending on their clinical condition. Patients who are HIV positive and have a CD4 count between 200 and 400 should receive 10cc subcutaneously daily for the first three days then 10cc subcutaneously every month for the next twelve months and should also be placed on the standard regimen for TB prophylaxis and other antibiotic antifungals according to the CDC guidelines. It is also possible to personalize a protocol for patients with particular debilitation from their disease.

HIV-1 infected patients have been treated with the VR-30 prepared as described in Example 3. Three male and one female HIV-1 infected patients, with viral burdens ranging from 43,000 to 9.4 million copies/mL received a series of three daily, s.c. injections of 10 mL VR-30 into the abdominal umbilical area, followed by a single, 10 mL injection of VR-30 administered monthly for up to an additional four cycles. Safety evaluations included
clinical evaluation, history and physical examination, and limited clinical pathology
evaluations, including complete blood counts, serum biochemistry, and CD4+ and CD8+ T
lymphocyte counts, as well as evaluation of serial HIV-1 viral burdens in two of the four
subjects.

All patients tolerated treatment with VR-30 well, with toxicities limited to local
irritation, itching, and redness, and pain at the site of injection. Increases in energy, appetite,
and weight gain were reported for all patients over the duration of treatment. There was no
evidence of systemic toxicity in these subjects; however, complete laboratory evaluations
were only available for one subject and no treatment-related changes in clinical pathology
parameters were observed, at up to 16 months following completion of VR-30 treatment.

Viral burdens were measured by HIV-1 RT-PCR assay in peripheral blood samples in
two patients treated with VR-30 in this study. One patient, who was naïve to antiretroviral
therapy, received a total of five cycles of VR-30 treatment over a five month period. Prior to
study treatment, the viral load in this subject was 9.4 x 10^6 copies of HIV-1 RNA/mL of
peripheral blood. Following the first cycle of three injections, the viral burden had decreased
to 1.1 x 10^6 copies/mL, and was further decreased following the third cycle of VR-30
injection to 230,000 copies of HIV-1 RNA/mL. However, the total number of peripheral
blood T cells, as well as the ratio of CD4+/CD8+ and the absolute CD4+ T cell counts
decreased over time in this subject, which is likely related to the lack of antiviral drug therapy
in this patient.

A second patient who had previously been treated with combination antiretroviral
drug therapy without significant response was injected with three doses of VR-30 over a
three-day period. This subject had a viral load prior to treatment of approximately 300,000
copies of HIV-1 RNA/mL. Six months following completion of the VR-30 injections, the
viral loads were undetectable (< 400 copies/mL, Roche Amplicor® HIV Monitor assay), and
serial samples in this subject remained below the limit of detection for this assay for an
additional 16 months. Both total T lymphocyte and CD4+ T cell counts were maintained or
slightly improved in this patient, with a value of 337 CD4+ T cells/mL blood in October of
2000, increasing to 508 CD4+ T cells/mL in November, 2002 at 16 months following
treatment with VR-30.

In summary, VR-30 injections were well-tolerated by the four HIV-1 infected subjects
treated with the composition of the present invention. There was no evidence of systemic
toxicity, and HIV-1 viral burdens were decreased in at least two of the subjects, including one patient who was naïve to antiretroviral drug therapy. These data suggest that VR-30 has potential anti-HIV viral activity.
Table 1: Inhibition of HIV-1 Viral Replication in Humans PBMCs by VR-30 Lot TV

<table>
<thead>
<tr>
<th>Dilution of VR-30 Tested</th>
<th>Mean Reverse Transcriptase Activity (cpm), ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Strain of HIV-1 Tested</td>
</tr>
<tr>
<td></td>
<td>BR/92/014</td>
</tr>
<tr>
<td>0</td>
<td>3452 ± 478</td>
</tr>
<tr>
<td>1:100,000</td>
<td>5490 ± 4237</td>
</tr>
<tr>
<td>1:32,000</td>
<td>6069 ± 1693</td>
</tr>
<tr>
<td>1:10,000</td>
<td>4616 ± 797</td>
</tr>
<tr>
<td>1:3200</td>
<td>5379 ± 2211</td>
</tr>
<tr>
<td>1:1000</td>
<td>5860 ± 2238</td>
</tr>
<tr>
<td>1:320</td>
<td>3101 ± 2748</td>
</tr>
<tr>
<td>1:100</td>
<td>4216 ± 218</td>
</tr>
<tr>
<td>1:32</td>
<td>3080 ± 856</td>
</tr>
<tr>
<td>1:10</td>
<td>5106 ± 3669</td>
</tr>
</tbody>
</table>

\[ a \text{ IC}_{50} = 1:320 \text{ dilution of VR-30} \]
\[ b \text{ IC}_{50} = 1:100 \text{ dilution of VR-30} \]

Table 2: Inhibition of HIV-1 Viral Replication in Humans PBMCs by VR-30 Lot HV

<table>
<thead>
<tr>
<th>Dilution of VR-30 Tested</th>
<th>Mean Reverse Transcriptase Activity (cpm), ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Strain of HIV-1 Tested</td>
</tr>
<tr>
<td></td>
<td>BR/92/014</td>
</tr>
<tr>
<td>0</td>
<td>3974 ± 1099</td>
</tr>
<tr>
<td>1:100,000</td>
<td>2067 ± 1692</td>
</tr>
<tr>
<td>1:32,000</td>
<td>4047 ± 2995</td>
</tr>
<tr>
<td>1:10,000</td>
<td>5753 ± 4349</td>
</tr>
<tr>
<td>1:3200</td>
<td>3607 ± 2297</td>
</tr>
<tr>
<td>1:1000</td>
<td>3868 ± 2473</td>
</tr>
<tr>
<td>1:320</td>
<td>6980 ± 2845</td>
</tr>
<tr>
<td>1:100</td>
<td>1984 ± 881a</td>
</tr>
<tr>
<td>1:32</td>
<td>2303 ± 225</td>
</tr>
<tr>
<td>1:10</td>
<td>1347 ± 831</td>
</tr>
</tbody>
</table>

\[ a \text{ IC}_{50} = 1:100 \text{ dilution of VR-30} \]
TABLE 3: Inhibition of HIV-1 Viral Replication in Human PBMCs

<table>
<thead>
<tr>
<th>Virus Strain</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; VR30 TV</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; VR30 HV</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; AZT</th>
</tr>
</thead>
<tbody>
<tr>
<td>BR/92/014</td>
<td>&gt;1:10</td>
<td>1:100</td>
<td>0.0001 µM</td>
</tr>
<tr>
<td>HT/92/593</td>
<td>&gt;1:10</td>
<td>&gt;1:10</td>
<td>0.0004 µM</td>
</tr>
<tr>
<td>HT/92/596</td>
<td>1:320</td>
<td>&gt;1:10</td>
<td>0.0009 µM</td>
</tr>
<tr>
<td>HT/92/599</td>
<td>1:100</td>
<td>&gt;1:10</td>
<td>&lt;0.0001 µM</td>
</tr>
</tbody>
</table>

TABLE 4: Inhibition of IV-1 Viral Attachment to CD4 Expressing Cells by VR-30

<table>
<thead>
<tr>
<th>Dilution of VR-30</th>
<th>Mean Luminescent Counts per Second, ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VR-30 HV Preparation</td>
</tr>
<tr>
<td>0</td>
<td>6440 ± 1275</td>
</tr>
<tr>
<td>1:1600</td>
<td>7281 ± 1189</td>
</tr>
<tr>
<td>1:500</td>
<td>7053 ± 965</td>
</tr>
<tr>
<td>1:160</td>
<td>6268 ± 1652</td>
</tr>
<tr>
<td>1:50</td>
<td>6551 ± 1526</td>
</tr>
<tr>
<td>1:16</td>
<td>5947 ± 2109</td>
</tr>
<tr>
<td>1:5</td>
<td>2556 ± 554</td>
</tr>
</tbody>
</table>

TABLE 5: Antibody Depletion VR-30

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>IC50 (DILUTION)</th>
<th>TC50 (DILUTION)</th>
<th>Therapeutic Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicago Sky Blue</td>
<td>1.3</td>
<td>&gt;10</td>
<td>&gt;7.7</td>
</tr>
<tr>
<td>(µg/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dextran Sulfate</td>
<td>0.65</td>
<td>&gt;25</td>
<td>&gt;38.5</td>
</tr>
<tr>
<td>(µg/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T.V. (IgG)</td>
<td>~1:25</td>
<td>&gt;1:10</td>
<td>---</td>
</tr>
<tr>
<td>T.V. (IgM)</td>
<td>~1:30</td>
<td>&gt;1:10</td>
<td>---</td>
</tr>
<tr>
<td>T.V. (IgG+IgM)</td>
<td>~1:20</td>
<td>&gt;1:10</td>
<td>---</td>
</tr>
<tr>
<td>T.V. (PBS)</td>
<td>~1:33</td>
<td>&gt;1:10</td>
<td>---</td>
</tr>
<tr>
<td>T.V. (Std Treatment)</td>
<td>~1:20</td>
<td>&gt;1:10</td>
<td>---</td>
</tr>
</tbody>
</table>

This invention has been described with reference to its preferred embodiments. Variations and modifications of the invention will be obvious to those skilled in the art from the foregoing detailed description of the invention.

60
1. A process for preparing a heterologous plasma cocktail which can be used to treat a host infected with human immunodeficiency virus (HIV), which comprises:
   a) exposing an animal to HIV which does not exhibit infection on such exposure;
   b) allowing time for the animal to respond to the virus and to produce one or more beneficial biologic agents in the blood;
   c) obtaining plasma from the animal; and
   d) using the plasma itself, with optional sterilization, dehydration, dilution or pharmaceutical preparation, but without further purification, to provide the heterologous plasma cocktail.

2. The process of claim 1, wherein the animal is a mammal.

3. The process of claim 2, wherein the animal is selected from the group consisting of cow, cat, dog, mouse, goat, lamb, sheep, horse, deer and pig.

4. The process of claim 1, wherein the animal is a goat.

5. The process according to claim 1, in which the plasma is obtained by:
   d) collecting the blood from the animal;
   e) treating the blood with an anticoagulant to prevent the formation of serum; and
   f) separating the plasma from the blood.

6. The process according to claim 5, in which the plasma is separated from the blood by centrifugation.

7. The process according to claim 1, in which the plasma cocktail is sterilized by filtration.

8. The process according to claim 1, in which the animal is an ungulate.

9. The process of claim 1, wherein the animal is allowed to respond to the HIV inoculation and to produce one or more beneficial biologic agents for between one and eight weeks.

10. The process of claim 9, wherein the animal is allowed to respond to the HIV inoculation for at least approximately three weeks.
11. A process for preparing a heterologous serum cocktail which can be used to treat a host infected with human immunodeficiency virus (HIV), which comprises:
   a) exposing an animal to HIV which does not exhibit infection on such exposure;
   b) allowing time for the animal to respond to the virus and to produce one or more beneficial biologic agents in the blood;
   c) obtaining serum from the animal; and
   d) using the serum itself, with optional sterilization, dehydration, dilution or pharmaceutical preparation, but without further purification, to provide the heterologous serum cocktail.

12. The process of claim 11, wherein the animal is a mammal.

13. The process of claim 12, wherein the animal is selected from the group consisting of ovine, cow, cat, dog, mouse, goat, lamb, sheep, horse, deer and pig.

14. The process of claim 11, wherein the animal is a goat.

15. The process according to claim 11, in which the serum is obtained by: collecting the blood from the animal, clotting the blood or allowing the blood to clot, and separating the clot from the serum.

16. The process according to claim 15, wherein the serum is clotted by adding clotting factor to the blood.

17. The process according to claim 16, wherein the clotting factor is selected from the group consisting of heparin, EDTA, citrate and oxalate.

18. The process according to claim 16, wherein the serum is clotting by contact activation.

19. The process according to claim 16, wherein the clot is separated from the serum by centrifugation.

20. The process according to claim 11, wherein the animal is an ungulate.

21. A pharmaceutical composition useful in the treatment of HIV which is prepared by:
   a) exposing an animal not susceptible to infection to HIV;
   b) allowing time for the animal to respond to the virus and to produce one or more beneficial biologic agents in the blood;
c) obtaining the plasma from the animal; and

d) providing the plasma itself, with optional sterilization, dehydration, dilution or pharmaceutical preparation, but without further purification, as the pharmaceutical composition for human administration.

22. The composition of claim 21 which is packaged as a pharmaceutical product.

23. The composition of claim 21 which is prepared as an intravenous formulation.

24. The composition of claim 21 which is prepared as an injectable formulation.

25. The composition of claim 21 which is prepared as a parenteral formulation.

26. The composition of claim 21 which is prepared as an oral formulation.

27. The composition of claim 21 which is provided as an intraarterial formulation.

28. The composition of claim 21, wherein the cocktail is sterilized by filtration.

29. The composition of claim 21, further comprising adding a pharmaceutically acceptable carrier or a diluent to the plasma.

30. A pharmaceutical composition useful in the treatment or prevention of HIV comprising unpurified plasma or serum from a goat immunized with HIV.

31. The composition of claim 20, wherein the goat plasma or serum has been sterilized by filtration.

32. A process for preparing a heterologous plasma or serum cocktail which can be used to treat a host infected with human immunodeficiency virus (HIV), which comprises:

a) exposing an animal to HIV which does not exhibit infection on such exposure;

b) allowing time for the animal to respond to the virus and to produce one or more beneficial biologic agents in the blood;

c) obtaining blood from the animal;

d) monitoring the blood for sufficient beneficial levels of one or more biological factors;

and

e) obtaining plasma or serum, with optional sterilization, dehydration, dilution or pharmaceutical preparation, but without further purification, to provide the heterologous plasma or serum cocktail.
33. The process of claim 32, in which the blood is monitored for the ability to inhibit HIV infection in vitro.

34. A method for the treatment of a patient infected with HIV comprising administering an effective amount of the heterologous plasma cocktail of claim 1.

35. A method for the treatment of a patient infected with HIV comprising administering an effective amount of the heterologous plasma cocktail of claim 4.

36. A method for the treatment of a patient infected with HIV comprising administering an effective amount of the heterologous cocktail of claim 11.

37. A method for the treatment of a patient infected with HIV comprising administering an effective amount of the heterologous cocktail of claim 14.

38. A method for the treatment of a patient infected with HIV comprising administering an effective amount of the heterologous cocktail of claim 21.


40. A method for the treatment of a patient infected with HIV comprising administering an effective amount of the heterologous cocktail of claim 24.

41. A pharmaceutical composition useful in the treatment of HIV which is prepared by:
   a) exposing an animal not susceptible to infection to HIV;
   b) allowing time for the animal to respond to the virus and to produce one or more beneficial biologic agents in the blood;
   c) obtaining the serum from the animal; and
   d) providing the serum itself, with optional sterilization, dehydration, dilution or pharmaceutical preparation, but without further purification, as the pharmaceutical composition for human administration.

42. A process for preparing a heterologous plasma or serum cocktail which can be used to treat a host infected with human immunodeficiency virus (HIV), which comprises:
   a) obtaining plasma or serum from an animal not susceptible to infection to HIV; and

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b) using the plasma or serum itself, with optional sterilization, dehydration, dilution or pharmaceutical preparation, but without further purification, to provide the heterologous plasma cocktail.

43. The process of claim 42, wherein the animal is a goat.

44. A pharmaceutical composition comprising the cocktail of claim 42 in combination with a pharmaceutically acceptable carrier.

45. The heterologous cocktail of claim 1, 4, 11, 14, 21, 23, 24 or 41 for use in medical therapy.

46. The heterologous cocktail of claim 1, 4, 11, 14, 21, 23, 24 or 41 for use in the treatment of a human infected with HIV.

47. The use of the heterologous cocktail of claim 1, 4, 11, 14, 21, 23, 24 or 41 for use in the manufacture of a medicament for the treatment of a patient infected with HIV.
INHIBITION OF HIV-1 HT/85/596 REPLICATION IN HUMAN PBMCs BY IMFTEX VR-30 (TV)

PERCENT OF CELL CONTROL

CONCENTRATION (ug/ml)

0.0069 0.001 0.01 0.101 0.32 1 3.16

% CC

% VC

PBMG Antiviral Assay - RT Endpoint

Figure 2