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Curing HIV-1 Infection via in vitro Ultra-Sensitive Modification of HIV-1 Uninfected $CD4^+$ Cells using Antiviral Agents

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Abstract
Curing HIV-1 Infection via *in vitro* ultra-sensitive modification of Human Immunodeficiency Virus-1 (HIV-1) uninfected $CD4^+$ cells is a promising hypothetical procedure that links different established clinical/laboratory tools (methods) and channeling them towards a clinical trial to achieve the ultimate goal of curing HIV-1 infection. The *In vitro* ultra-sensitive chemical modification of HIV-1 uninfected $CD4^+$ cells using selected anti-viral agents towards curing HIV-1 infection entails a six step clinical/laboratory procedure. Isolation/purification of HIV-free $CD4^+$ cells is the first step of this therapeutic procedure. *In vitro* $CD4^+$ cell expansion/culture, ultra-sensitive chemical modification, inhibition/suppression of $CD4^+$ cell proliferation, administration of chemically modulated cells to HIV-infected patient, monitoring/maintaining the physiological/biochemical processes under intensive care make up the other steps of this procedure consecutively. The milestones in this therapeutic method include increased bioavailability, minimum dosage, minimal or no side effects, shorter duration of treatment, mop-up of free plasma virions. The principal reason for this adopted method is that, the therapy overcomes the limitations posed by the current methods of managing HIV-1 infection using anti-retrovirals.
Keywords: Curing, $CD4^+$ cells, HIV-1 infection, antiviral, side effect, modification

1.0 Introduction

Viruses are the most notorious of all pathogenic organisms of humans, and we lack foolproof anti-viral therapies against them (Shailendra *et al.,* 2009). So far, most of the antiviral agents presently employed in managing HIV-1 infection come with side effects that to some extent offset their therapeutic benefits (Monica, 2009). HIV-1 infection when untreated results in a progressive destruction of the immune system resulting in acquired immunodeficiency syndrome (AIDS) (Boasso *et al.* 2009) defined as the presence of HIV infection with a CD4 cell count less than 200 cells/mm^3 (Ali, 2007). HIV/AIDS continues to be a major and one of the leading diseases with an estimated 2.7 million new infections in 2007 and an estimated 2 million deaths was recorded following HIV-related illnesses in the same year (Nick, 2009). HIV was recovered first from infected cluster of differentiation positive ($CD4^+$) lymphocytes and was considered a T-lymphotropic virus (Levy, 1993). Subsequent studies indicated a wider host range for this virus, including macrophages, other hematopoietic cells, CD4-negative human fibroblasts and brain-derived cells (Levy, 1993). However, replication occurs at its highest titer in $CD4^+$ lymphocytes (Shenbei *et al.* 1995). The CD4 T (helper) are the coordinators of the body's immune response. The "CD" or cluster of differentiation is a protein expressed on the surface of the cells of the hematopoietic system and within hours of exposure to HIV, $CD4^+$ T lymphocytes are found to be infected showing active viral replication (Alan *et al.* 1996). Millions of $CD4^+$ T lymphocytes may be destroyed every day, eventually overwhelming the immune system's regenerative capacity. Although differences exist in the range of values for normal CD4 count obtained from various laboratories/clinics, a normal CD4 count has a range of 600–1,500 cells/mm^3. In HIV-1 infection, $CD4^+$ T cells specific for HIV-1 are infected by the virus at higher frequencies than other memory $CD4^+$ T cells. HIV-1 specific $CD4^+$ T cells are barely detectable in most infected individuals and the corresponding $CD4^+$ T cells exhibit an immature phenotype compared to both cytomegalovirus (CMV)-specific $CD4^+$ T cells and other memory $CD4^+$ T cells (Jason *et al.* 2006). The *in vitro* ultra-sensitive modification of HIV-1 uninfected $CD4^+$ cells using antiviral agents is aimed at presenting a step by step clinical/laboratory procedure as part of a cycle of repeated process towards curing HIV-1 infection.

1.1 Milestones in this therapeutic procedure

It's vital to know that most of the side effects of antiretroviral medications originate from the dosage and route of administration. Some of the most notable side effects of antiretroviral drugs are diarrhoea, nausea and vomiting, rash, lipodystrophy, lipid abnormalities and myocardiac infarction. A study review has found that 60 percent of people on antiretroviral drugs report diarrhoea (MacArthur *et al.* 2012), most especially during the first few weeks of treatment and can reduce appetite (AVERTing HIV and AIDS, 2014). This may generate more serious problems such as pancreatitis or lactic acidiosis. Rashes often appear as a side effect of antiretroviral treatment and often itchy but are usually harmless and short-lived. Lipodystrophy involves losing or gaining body fat,

often in ways that can be disfiguring and may be in the form of losing fat on the face, arms, legs and buttocks, resulting in sunken cheeks, prominent veins on the limbs, and shrunken buttocks (AVERTing HIV and AIDS, 2014). HIV positive patients taking antiretroviral treatment commonly have high levels of a lipid called LDL cholesterol, low levels of HDL cholesterol, and high levels of triglyceride in the blood (AVERTing HIV and AIDS, 2014). In 2008, a large investigation called the D: A: D study reported that the use of the NRTI abacavir was associated with a higher risk of myocardial infarction compared to other drugs in the same class (D: A: D Study Group *et al.* 2008).

Bioavailability is a subsection of absorption of medication; the portion of an administered dosage of unaltered drug that gets to the systemic circulation for further metabolism. When a medication/drug is administered intravenously, its bioavailability is 100% (Griffin, 2009). However, when a medication is administered via other routes (most especially orally), its bioavailability generally decreases due to incomplete absorption and first-pass metabolism (Mukonzo *et al.* 2011). For polar compounds of which antiretroviral agents are a part of, the major factor restricting their entry lies in the tight junctions that occlude the paracellular pathway across these barriers. In addition, influx and efflux transport systems, or metabolic processes active in both capillary endothelial cells and choroid plexus epithelial cells, can greatly change the bioavailability of a drug in one or several compartments of the CNS (Strazielle & Ghersi-Egea, 2005). Other major factors that may affect drug availability are Enzyme Induction and Inhibition, Enzyme Polymorphisms, and Disease States (Romil, 2011). The physicochemical factors affecting drug absorption include the pH-partition hypothesis on drug absorption, ionization and pH at absorption site, lipid solubility (Wolters, 2012).

Antiretroviral agents mostly exert their toxic, carcinogenic, allergic and mutagenic effects by going to other cells, tissues or organs that are not the target. The *in vitro* Ultra-sensitive Modification of HIV-1 Uninfected $CD4^+$ Cells however, is set out to boycott all these limitations since drug delivery is *in vitro*. The highest level of drug targeting expressed here will reduce all side effects to the minimum. This is evident in the minimal dosage of anti-viral agent, and the duration of treatment will definitely be shorter. The patient might be cured in a matter of days or few weeks. A remarkable feature of this method is also the mop-up of free plasma virions.

1.2.1 Isolation/purification of HIV-1 uninfected $CD4^+$ cells (step 1)

Human $CD4^+$ lymphocytes are heterogeneous and can be distinguished by cell surface proteins that denote adhesion and activation antigens. This step of the procedure is aimed at isolating a set of HIV-1 uninfected cells from the source. Choice of HIV-free $CD4^+$ cells donor could be presumptuously achieved in two ways.

(a) The beneficiary (HIV infected patient) will be the donor. Making this choice will require a series of clinical tests to distinguish HIV infected $CD4^+$ cells from non-infected ones.

(b) The donor will be HIV-seronegative individual and must not necessarily be the beneficiary.

Compatibility check to ensure a close match between the donor and the recipient should be ordered to minimize immunological complications.

The advantage of option (a) over (b) is that complications that could probably arise from immunological responses of self against non-self (foreign body) is eliminated.

A number of methods can be employed in achieving the isolation and purification of uninfected $CD4^+$ cells. Nakamura *et al.* demonstrated that, Human PBMC isolation is achievable by immunomagnetic beads through Ficoll/Conray gradient centrifugation [18]. Thereafter, the separation of $CD4^+$ T cells can be performed by a positive selection technique as reported by Gaudernack *et al.* using sheep anti-mouse IgG-coated immunomagnetic beads (Gaudernack *et al.* 1986). The purity of $CD4^+$ T cells in recovered cells was over 98%. The isolated CD4+ T cells consisted of 46% of CD45RO and 54% of CD45RA. Through adherence to plastic, $CD4^+$ Peripheral blood mononuclear cells (PBMC) could also be obtained by Ficoll-Hypaque separation of peripheral blood buffy coat as well as Peripheral blood macrophages from these PBMC (Homsy *et al.* 1989).

Invitrogen life technologies provides a viable method of a high yield of pure $CD4^+$ Isolation from whole blood, buffy coat, mononuclear cells (MNCs), or bone marrow (Life Technologies, 2011). Invitrogen Corporation also provides a viable negative cell isolation technology for isolating pure and untouched human $CD4^+$ T cells (Invitrogen Corporation, 2008) where an antibody mix is added to bind to non-$CD4^+$ T cells (B cells, NK cells, monocytes, platelets, dendritic cells, $CD8^+$ T cells, granulocytes, and erythrocytes). Dynabeads® then bind to these antibody-labeled cells and with the aid of a DynaMag™ magnet, the bead-bound cells are captured and discarded.

Miltenyi offers another method for the purification of cells from several organisms including humans, non-human primates, rat and mice. This magnetic bead-based cell separation allows for either positive selection (or cell depletion) as well as negative selection. Employing this standard protocol for the purification of cells guarantees 96% pure $CD4^+$ cells (Matheu & Cahalan, 2009).

James, LaFond, Durinovic-Bello and Kwok, provided a detailed description of a method of isolating $CD4^+$ cells from PBMC, in which blood is collected in syringes or blood tubes and anti-coagulated with heparin (1:50 ratio)

to prevent clotting (James *et al.* 2009). Expected yield is about 1×10^6 PBMC per mL of blood – about 40% of which will be $CD4^+$ T cells. Thereafter, the succeeding steps in the isolation process are carried out. The cell counts at the end of the isolation process will dictate the number of wells used for the expansion culture step when required.

1.2.2 In-vitro CD4+ cell culture/expansion (step 2)

It has been demonstrated that the culture of peripheral blood mononuclear cells (PBMC) in the presence of interleukin 2 (IL-2) caused the predominant growth of CD8+ T cells (Taylor *et al.* 1985) and the selective *in vitro* growth of CD4+ T cells in the presence of IL-2 has been considered to be difficult. The different IL-2 responsiveness of CD8+ and CD4+ T cells was demonstrated to be derived from their differential expression of p75 IL-2 receptor (IL-2R) (Nakamura *et al.* 1991). It has been however, demonstrated that stimulation of FACStar-sorted CD4+ T cells with immobilized OKT-3 monoclonal antibody (mAb) induced p75 IL-2R expression and IL-2 responsiveness of CD4+ T cells. Moreover, CD4+ T cells have demonstrated to display both IL-2 producing activity and bispecific antibody-directed antitumor activity (Nishimura *et al.* 1992). A simple method for the isolation and expansion of CD4+ T cells is described by a simple method for the generation and expansion of CD4+ T cells with both helper and killer functions in the presence of immobilized anti-CD3 mAb plus IL-2 [18]. The large-scale expansion of activated CD4+ helper/killer T cells was achieved using a concentrated rotary tissue-culture (CRTC) bag alongside the in vitro targeting of the CD4+ helper/killer T cells by means of anti-CD3 x anti-c-erbB-2 bispecific antibody (BsAb). Large-scale expansion of CD4+ T cells can be performed by culturing with immobilized OKT-3 mAb plus rIL-2 (Nakamura *et al.* 1992). This large scale culture yielded an approximate 3000-fold increase in cell numbers after 16 day-culture. Dynal Biotech provides a putative efficient method of cell culture. Over 1000 fold expansion of T cells can be achieved in 12 days when naïve T cells are incubated with Dynabeads® CD3/CD28 T Cell Expander (Dynal Biotech, 2002). James, LaFond, Durinovic-Bello and Kwok, provided a description of a method of expansion culture of CD4+ cells in vitro that follows the isolation of CD4+ cells from Peripheral blood mononuclear cell (PBMC) (James *et al.* 2009) as described in the previous section.

1.2.3 Ultra-sensitive Chemical modification of the clone using antiviral agent (step 3)

The *in vitro* chemical modification involve incubating the clone of $CD4^+$ cells in a physiological medium containing the right concentration of the anti-viral agent. The anti-viral agent is transported across the membrane to the site of action. The therapist should be able to use available newer, more potent, and less toxic antiretroviral (ARV) agents for this trial. Effective concentration (EC_{50} and EC_{90}) at which HIV-1 replication is inhibited in the CD4+ cell must be established. The drug delivery system employed plays a vital role in controlling the pharmacological effect of the drug as it can influence the pharmacokinetic profile of the drug, the rate of drug release, the drug bioavailability, the site and duration of drug action and subsequently the side effect profile (Yvonne & Thomas, 2010). We therefore, project an *in vitro* delivery of the therapeutic antiviral agents in to the clone of CD4+ cells prior to administration of the $CD4^+$ cells to the patient. This will ensure an optimal drug delivery system in which the active drug is available at the site of action at the correct time and duration.

1.2.4 Inhibition/suppression of $CD4^+$ cell proliferation in the HIV-infected patient (step 4)

The patient is placed under intensive care in a sterile environment. This is because the suppression of the activity of the $CD4^+$ cells that mediate the cell-mediated immunity will make the patient vulnerable to other infectious organisms. The sole aim of suppressing the further production of more $CD4^+$ cells is to halt the production of cells that lack the therapeutic modification described in the step above, which may serve as factories for HIV-1 proliferation. A number of immunosuppressive drugs are available and may include corticosteroids, purine pathway inhibitors such as azathioprine and mycophenolate, pyrimidine pathway inhibitors, immunophilin-binding agents and alkylating agents (Lee & Andrew, 2012).

1.2.5 Administration and activation of the chemically modulated clone to the infected patient (step 5)

The route of administration of the *in vitro* chemically-modulated set of CD4+ cells will be intravenous: small volumes can be given as a single dose whereas larger volumes can be given by infusion. A big question here is about the quantity of chemically modified $CD4^+$ cells that will be administered to patients. HIV-1 replication *in vivo* occurs continuously at high rates (Alan *et al.* 1996) and according to Alan *et al.* the estimated average total HIV-1 production was 10.3×10^9 virions per day. Alan *et al.* used a mathematical model to explain HIV-1 dynamics *in vivo* (Alan *et al.* 1996). A potent HIV-1 protease inhibitor, ritonavir was administered orally to the patients (600 mg twice daily) through the period of investigation and the result of the research is an essential component of the bed rock of this proposed therapy (Alan *et al.* 1996). Administered ultra-sensitive chemically modified CD4+ cells are readily available to be infected by free virions, but will however, be preceded by the commencement of treatment with a potent HIV-1 protease inhibitor such as ritonavir or more potent ones. Cell

death could be induced at this stage in the administered cell 2.6 days (62 hours) after administration as a therapeutic measure. HIV-1 generation time-defined as the time from release of virion until it infects another cell and causes the release of a new generation of new particles is 2.6 days (Alan *et al.* 1996). Therefore, in order to halt any likelihood of release of new generation of viral particles that are products of the complete life cycle of virions that survived the effect of the anti-viral agent such as alkylating antineoplastic agent as well as the protease inhibitor, we induce cell death in the administered modified cells. This is then followed by the administration of a new dose of modified cells. However, the minimum duration of HIV-1 *in vivo* is 1.2 days (28 hours) on average (Alan *et al.* 1996).

Knowing that the estimated average life span of productively infected cells is 2.2 days (52 hours) (Alan *et al.* 1996), a new dose of the modified cells can be administered after 52 hours. We presume that after about 52 hours, the previously administered dose of modified cells would have been killed by invaded viruses. It is important to note that this method is an alternative to the first method (induction of cell death after 62 hours) described in the previous paragraph. Though there are a lot of irregularity in getting a universally regular normal range of values for CD4 count, we 600-1500 cells/mm^3 is often used by many standard laboratories. The quantity of CD4+ cells to be administered to the patient will depend on the stage of the infection in the patient and the outcome of the routine test at regular intervals described previously. However, we propose a formula as depicted below that could make the work of the therapist easier.

$$v = (x \times z)/y$$

x is the small volume of choice taken from the stock of $CD4^+$ culture medium stock

y is the number of $CD4^+$ cells gotten from a CD4 count performed on x

v is the volume of $CD4^+$ cells medium to be administered to the patient

z is the number of CD4 cells to be administered to patient.

z is to be decided by the therapist after a CD4 count is carried out for the patient. However, there are there are two alternatives here. The therapist could decide to administer a complete volume that will supply a complete number of CD4 count regardless of the level of the CD4 cells in the patient before administration. Alternatively, with regards to the level of CD4 cells in the patient, the therapist could administer a volume that will make up the initial level to the normal level.

1.2.6 Monitoring and maintaining the physiological/biochemical processes (step 6)

In addition to the series of other successive routine clinical tests, HIV RNA (viral load) and CD4 T lymphocyte (CD4) cell count will be the two surrogate markers of treatment responses and HIV disease progression. Viral load is a marker of response to ART to provide prognostic information about the probability of disease progression (Murray *et al.* 1999). Viral load is the most important indicator of initial and sustained response to ART. Several systematic reviews of data from clinical trials involving thousands of participants have established that decreases in viral load following initiation of ART are associated with reduced risk of progression to AIDS or death (Thiebaut *et al.* 2000). There are a number of recommendations on the frequency of viral load monitoring depending on situations such as, initiation of ART or modification of therapy because of virologic failure, virologically suppressed patients in whom ART was modified because of drug toxicity or for regimen simplification, patients on a stable suppressive ARV regimen, and patients with suboptimal response. This review however, suggests that the frequency will be determined by the result of the previous test while the patient is kept under intensive care. The CD4 count is the most important laboratory indicator of immune function in HIV-infected patients. The CD4 cell count provides information on the overall immune function of an HIV-infected patient. It is also the strongest predictor of subsequent disease progression and survival according to findings from clinical trials and cohort studies (Egger *et al.* 2002). Splenectomy (Bernard *et al.* 1998) or co-infection with human T-lymphotropic virus type I (HTLV-1) and other factors may cause misleadingly elevated CD4 counts. Alpha-interferon may reduce the absolute CD4 count without changing the CD4 percentage (Casseb *et al.* 2007). In all these settings, CD4 percentage remains stable and may be a more appropriate parameter to assess a patient's immune function. These tests are critical in establishing thresholds for the initiation, continuation and discontinuation of the administration of chemically modified CD4+ cells. These tests should be done at initiation of therapy and at regular intervals as well as at other intervals considered fit by the therapist and may not necessarily be regular. It's vital to note that the patient is kept under intensive care in a sterile environment throughout the trial.

Conclusion

The side effects that accompany most of the antiretroviral agents sometimes outweigh their therapeutic effects. The stress of repeated regimented dosage as well as the chronic duration of treatment can be tedious for the patient. It is therefore pertinent that we look for a faster, more efficient and safer method of managing HIV/AIDS infection. Taking this clinical procedure further to clinical trial stage will definitely push us further

towards achieving the goal of curing HIV/AIDS infection that the health community has long struggled with.

Conflict of interest

Authors wish to notify you that there is no conflict of interest in this research

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