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Preliminary findings in cure of two HAART experienced HIV patients by stopping reverse dissemination from bone marrow CD4 progenitors

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Abstract: Elucidation of reverse dissemination as the true mechanism by which HIV maintains chronic infection while discounting of the generally accepted model of latent reserve can open a new frontier in research that could result in radical cure. HIV may cause and maintain chronic infection by reverse disseminating from differentiating CD4 T-lymphocyte progenitor cells (LPCs) within the bone marrow niche, and that breaking the cross-infection between older and new cells can lead to elimination of the reserve infection result in radical cure. By using a mechanism that prevents the rapid expansion of HSCs that give rise to LPCs, I collected data and information from two patients that show achievement of radical cure of HIV by absence of viral resurgence for eight months after stoppage of highly active antiretroviral therapy.

Keywords: HIV, Reserve Infection, Reverses Dissemination, Radical Cure

1. Introduction

Hematopoietic stem cells (HSCs) give rise to hematopoietic progenitor cells (HPCs), which are multipotent cells that differentiate to all the blood cell types from the myeloid (monocytes and macrophages, neutrophils, basophils, eosinophils, erythrocytes, megakaryocytic/platelets, dendritic cells), and lymphoid lineages (T-cells, B-cells, NK-cells). The hematopoietic tissue contains cells with long-term and short-term regeneration capacities and committed multipotent, oligopotent, and unipotent progenitors. HSCs constitute 1:10.000 of cells in myeloid tissue. HSCs are defined by their ability to replenish all blood cell types (multipotency) and their ability to self-renew.

A small number of HSCs can expand to generate a very large number of daughter HSCs. This phenomenon is used in bone marrow transplantation where a small number of HSCs are used to reconstitute the whole hematopoietic system. Stem cell self-renewal is thought to occur in the stem cell niche in the bone marrow and it is reasonable to assume that key signals, together with environmental and molecular requirements present in this niche, will be determine the process of self-renewal.[2] Some of the factors that influence the renewal environment may be directly linked to particular hematopoietic progenitor cell demand by the organism, as this does not occur spontaneously *in vitro*. Progenitor cell demand would in turn be influenced by demand of the particular cell by the whole system of the organism. Of much interest is the ability of HSCs and some primitive progenitor cells to migrate from the bone marrow into general circulation. However, once progenitor cells reach a certain level of differential development, they are prevented from entering general circulation until they reach maturity.

HSCs are a heterogeneous population but HPCs become committed to a lineage at some stage during development.[2] Studies have shown that CD4 is expressed in 25%–65% of $CD34^+$ bone marrow cells, and these studies have estimated that the level of expression is half of what has been observed in monocytes and only 5% of the level reported on $CD4^+$ T cells.[3, 4.] This commitment results in acquisition of unique characteristics for the cells following a particular lineage, and it occurs in distinct, characterized stages. Committed HPCs lose pluripotency, lack the capacity for self-renewal, have a higher fraction of cells traversing the cell cycle, and a change in their surface protein profile.[5] This means committed HPCs must proceed in development to full maturity and leave the bone marrow niche to perform their functions elsewhere (except megakaryocytes

which remain in bone marrow after full development). HSCs are able to give rise to common lymphoid progenitors (CLPs) and common myeloid progenitors (CMPs). CLPs and CMPs can develop into any of the lymphoid and myeloid lineages respectively, depending on the particular cell demand by the general system. Production can be skewed towards the cell type to which demand is highest and could probably preferentially favor the cell type considered more essential, as evidenced by increased homing of CD4 lymphocytes into bone marrow in HIV infection compared to non infected individuals.[6] The T cell is a critical component of cellular immunity and can be considered above the other lineages in the event of production demand. This is why despite being the principal target cell in retroviral disease, T cell levels are maintained even at the expense other target cells are which become relatively depleted.

It has been recently suggested that HIV maintains chronic infection by reserve infection sequestered in the bone marrow niche, where it is avoids cellular immunity that would otherwise eliminate it.[7] It means for chronic HIV infection, this reserve infection must be maintained by continuous replication to full viral cycle and production new virus progeny for the continuous infection of new cells within the niche as they develop, while the older cells are either killed or leave the niche altogether. This is opposed to the proposition that the virus remains dormant within host cell in the form of provirus and activates to full cycle replication at some stage in the future.

HSCs and the most primitive hematopoietic progenitor cells (HPCs) are resistant to HIV-1 infection.[8] However, once these cells begin to differentiate and become committed HPCs (particularly T- lymphocyte developmental stages), they become increasingly susceptible to HIV-1 infection and permissive to viral gene expression and infectious virus production.[9] Trafficking of bone marrow-derived HIV-1-infected monocytes has been shown to be involved in the dissemination of HIV-1 into the central nervous system (CNS),[10] and it is possible that HIV-1 replication in bone marrow HPCs may be involved in the early steps leading to the development of HIV-1-associated dementia (HAD) as an end result of this cellular trafficking process. In addition, the growth and development of HPCs in the bone marrow of patients with HIV-1 has been shown to be impaired due to the presence of HIV-1 proteins and changes in the cytokine milieu, with the alteration of maturation process and increased cell death within the cell lineages.[9, 11-13] Defects within the progenitor cell populations of patients with HIV-1 are well documented with evidence showing relative depletion of primitive $CD34^{+}CD4^{+}$ progenitor cells compared to healthy controls. This shows that the suppression of myelopoiesis occurs early in the course of retroviral disease and the mechanism of HIV-induced myelosuppression may be due to the tremendous strain on resources directed at replenishing diminishing lymphocytogenic HPCs (in the face of heightened demand of CD4 lymphocytes by the system) rather than any direct viral action on other susceptible or non-susceptible cells.

Although cellular immunity can eliminate HIV from the general body, it could be absent in the bone marrow niche where HPC development occurs.[7] This means infection of a lineage of HPCs in the bone marrow creates a stable, self replenishing reserve. As new target cells continuously and increasingly develop from HSCs to meet demand, they are subsequently infected by virus from older cells that become productive in the niche. Virus leakage into circulation as well as cell trafficking ensures other expendable reserves elsewhere in the body are maintained and continuously increased. This situation is possible due to high hematopoietic cell turnover, increased especially in HIV infection because of feedback production occasioned by viral and immune killing of target cells heightening demand. It appears that even when there is effective antiretroviral therapy, continuous infection of newly differentiated CD4 lymphocytogenic HPCs is maintained though probably to a much lesser extent than is in the case otherwise. It is reasonable to assume that not all susceptible differentiating HPCs in the lineage may get infected though, meaning some are able to leave the niche as normal cells and may get infected later while in circulation or might be protected to form part of the normal uninfected count in circulation. In the advent of antiretroviral therapy (ART), infection occurring in circulation is considerably reduced due to lower viral loads, allowing steady CD4 count recovery and maintenance.

Other stromal cells are also infected. Among the stromal cells, macrophages are the most prominent cell type that are productively infected with HIV-1 and express viral antigens.[14] Macrophages in the bone marrow have been shown to be susceptible to both macrophage tropic and T cell–tropic strains of HIV-1, and to have a broader susceptibility to HIV-1 strains than blood-derived macrophages.[15, 16] Eosinophils differentiated in vivo from bone marrow stromal cells, and bone marrow fibroblasts, are also permissive for HIV-1 infection.[17,18] However, these may not constitute the critical reserve infection as they are not sequestered from cellular immunity, have a significantly much lower cell turnover and are, as for macrophages, mobile between bone marrow niche and general circulation. It is possible that stromal cells within the bone marrow may not even be part of the niche. Apparently, this group and others such as bone marrow microvascular endothelial cells and megakaryocytes, are infected mostly opportunistically due to prevailing high viremia and cannot maintain infection if the source of viremia is removed.[7] Megakaryocytes, for example, although they remain in the bone marrow following maturation, have been shown to get depleted early in the course of HIV infection leading to thrombocytopenia,[19] probably because they lack the benefit of high turnover as is the case for CD4 lymphocytes. This also means they cannot be responsible for chronic maintenance of HIV. Although eosinophils and fibroblasts are permissive to

HIV-1 infection, they are not significantly productively infected and may probably represent dead end hosts to HIV, being incapable of propagating the virus in any manner that can sustain chronic infection or taking considerably long time to reach viral production. Infected macrophages on the other hand are highly productive, coalescing and spewing large amounts of virus, but they are also extensively damaged and invariably lose their function and die. Macrophages play an important role in the dissemination of HIV in the brain and other secondary tissues through trafficking and not maintenance of a self-replenishing reserve. Because of low turnover rate of macrophages and their mobility between bone marrow compartment and the general circulation, they cannot form the sustainable reserve infection of the kind that is formed by T-lymphocytogenic HPCs confined in bone marrow niche.

2. Hypothesis

2.1. Effective Infection by HIV Leading To Retroviral Disease

For HIV infection to occur there must be effective transmission from an infected source to the subject. It is agreed that this transmission occurs by body fluid contact involving the general circulation of the subject.[20, 21] Transmission itself however, does not always result in infection. Transmissible HIV is in the form of free virus that is capable of entry into the subject host cell system and proceeding to form productive infection. Transmission may also occur as transfer of infected host cells but which must be capable of viral production themselves. Free viral RNA cannot cause infection. Neither can proviral DNA confined in the nucleus of a host cell without vegetative viral production. A single virus particle attacking a lymphocyte, for example, may be incapable of causing productive infection of a susceptible target cell even upon successful entry. This is because the cellular viral replication process depends on accumulation of viral proteins[22] for its own purposes (such as nef, for translocation of unspliced mRNA and tat, for new virus formation), and for the purposes of defeating host innate defenses (such as vif, APOBEC system[23]). Because target cells such as CD4 lymphocytes have a limited life span, the process of accumulation of viral proteins must be fast enough to achieve viral production before the end of the life of the cell, and besides an infected cell might soon be destroyed by the immune system or apoptosis. This means therefore that productive infection is only possible upon successful entry by a large number of virus particles. This should explain the observed critical role of CD4- CCR5 receptor complex in transmission of HIV despite the presence of other receptor systems. But this race against time favors the virus in the bone marrow niche, due to absence of cellular immunity and the fact that the differentiating T-lymphocytes are highly susceptible and must extend their stay in the niche as programmed. Outside of the bone marrow niche, the situation is different, as can be seen at the onset of specific immunity during primary HIV infection. At this stage the viremia is very high, to the tune of several million virus particles per milliliter of blood; specific immunity breaks the replication cycle by killing infected cells before new particles can be formed and manages to control the viremia to relatively low levels of about four hundred thousand particles per milliliter. It has been in fact suggested that this immune action alone can eliminate HIV summarily in the absence of reserve infection sequestered in the bone marrow.[7]

Effective HIV transmission requires the presence of highly susceptible target cells at the point of entry. These cells are the CD4 T-lymphocytes as they are the ones expressing high numbers of $CD4^{+}CCR5$ receptor complex necessary for the rapid entry and subsequent viral replication to provide the populations required for effective primary dissemination. Dissemination leads to primary disease where the virus accesses large populations of the primary target cells as well as other less susceptible secondary targets (macrophages, dendrite cells) and secluded tertiary systems (bone marrow niche). Once the bone marrow CD4 lymphocytogenic niche is infected, the virus is able to reverse-disseminate from this site, and so to maintain waning infection in secondary reservoirs as well as the general circulation. Soon, the primary target cells in circulation are protected by immune response and viremia is mainly maintained by longer lasting secondary targets with reduced participation of circulating lymphocytes. With time, the secondary targets are increased by involvement of more cells forming what is called HIV reservoirs. The involvement of the bone marrow CD4 lymphocytogenic niche probably starts during the peak of primary infection and is possible by viral trafficking by infected CD4 cells homing into the niche and discharging their load therein, therefore seeding the niche. Because of very high feedback production of the primary target cells and absence of cellular immunity, viral production in the niche is efficient and viral leakage from here can reverse disseminate to bolster waning populations in circulation and secondary tissues. Although viral load levels are controlled by onset of sero-conversion and it is expected that bone marrow niche involvement should remain constant, situations in the course of the retroviral disease may cause periodic viral load escalations that can lead to fresh seeding of more niche units as they are produced, leading to greater bone marrow involvement and higher level of reserve infection. Such situations may include immune-demanding co-infections, malnutrition and probably cluster maturation of slower reservoirs into viral production, among others. Eventually the niche-forming capacity of the bone marrow will be exhausted, which may be why some HIV patients at the very near death end never recover their CD4 count even when on effective HAART.

Primary target cells provide rapid viral production necessary for the transmission and dissemination of HIV by expressing abundant $CD4^{+}CCR5T$ receptor complex on the

surface, allowing multiple viral entry and accelerated viral processes as opposed to the much slower secondary reservoir cells. The $CD4^+CCR5$ receptor complex is therefore critical for the transmission, dissemination and reverse dissemination of HIV. Studies have shown that elimination of this receptor system leads to HIV cure, even in the presence of other receptor systems.[24] Studies also suggest that other receptor systems are of low efficiency and get involved much later in the course of HIV disease, [25] more as an opportunistic consequence of persistent viremia maintained by the $CD4^+CCR5$ system. Removal of the $CD4^+CCR5$ system renders all other entry systems destitute, unable to allow productive infection that is fast enough to compete with the body's innate defense, and therefore unable to maintain chronic HIV infection or probably even transmission.

2.2. Pathogenesis of Retroviral Disease

The burden of replenishing CD4 T-lymphocytes under viral attack, the cost of maintaining activated specific immune response to HIV, and the challenges arising from accumulation of cellular and metabolite waste of these processes, are the causes of the progression sequels of retroviral disease rather than the depletion of the CD4 cells themselves. To start with, although primary HIV infection causes very high viremia and rapid fall in levels of CD4 T-lymphocytes, AIDS does not set in then. The onset of specific response at sero-conversion controls viremia and allows CD4 replenishment through increased hematopoietic production and protection of circulating cells and the primary stage passes largely unnoticed.

In reference to the general trends of HIV viral loads and CD4 profile in the progression of untreated retroviral disease illustrated in the graph below sourced from Wikipedia and which is generally accepted as the a fairly accurate representation events occurring in the retroviral disease (figure 1).

At the onset of primary infection, viral loads peak to around one million copies HIV RNA per mL (cpm) plasma by week 6. This coincides with a similar and rapid fall in CD4 lymphocyte count to a low of about 450 cells per mm3 of plasma. It is logical to relate that the initial increase in viremia in primary infection to the availability of highly susceptible primary cell targets, i.e., the CD4 lymphocytes, without which this increase would not be possible. It is the peaking of viremia at six weeks is responsible for the dissemination of the virus throughout the body and this is possible because of availability of the highly susceptible primary target cells.

While the viral load decrease following initial peaking is maintained at an almost constant low of about 350 cpm, the CD4 count actually rises significantly to about 700. This implies that the primary target for HIV, though still present in sufficient quantities, is somehow protected from the wanton attack of the same degree seen during the initial period. Indeed this period coincides with sero-conversion, or the onset of a specific immune response to HIV, a situation which leads to protection of circulating CD4 lymphocytes from the kind of brazen attack seen at the opening of primary infection. This protection is, however not absolute, as the attack will continue, albeit at much lower rates. Viremia at this stage may be maintained principally via supply of virus disseminated from secondary and tertiary reserves rather than virus derived from circulating CD4 cells themselves; otherwise we would have seen continued depletion of the CD4 count to much lower levels.

Over many years, starting at about week 10, the viremia is maintained at low levels ranging between 300 and 500 cpm with a general marginal increase over the span of about 10 years, while the CD4 count decreases over the same period at a higher rate when compared to the increase in viral loads. This suggests that the reason for the decrease in CD4 count is not the plasma viremia alone; other factors must be in play as well. Indications that this decrease may be initially due to increasing viral reserve in both the secondary tissues and greater involvement of the bone marrow niche probably due to periodic viral load peaks need to be investigated. Indeed it is known that good nutrition delays the onset of AIDS, probably by maintaining the supplies needed to keep up CD4 production at the niche and nourishing the rest of the immune response. This would delay the immune-suppression caused by HIV which probably occurs due to depletion of peripheral supplies by the over-demanding niche. The over-demanding niche, in theory, may be the true cause of AIDS by causing malnutrition in the general body thus causing the metabolism to turn catabolic, leading to wasting and accumulation of waste products and systems breakdown. The main challenge appears to be the maintenance of supplies to meet the demand at the niche while supplying the regular requirements of the body as well, and clearing the system of the tremendous waste that results from the viral activity and the catabolic activity. This association has in the past fuelled significant AIDS denialism.[26- 28]

The viral loads increase steadily and slowly up to about 9 years, and then suddenly surge upward over the last year in spite of the decreasing and low CD4 count. This means the increase is not related to availability of CD4 cells in circulation alone. The increase may be initially due to increasing viral leakage from the niche as the niche capacity expands to increase CD4 production, basically, increased reverse dissemination from the niche into circulation. At the onset of AIDS, the increase is much sharper because immune response responsible for controlling plasma derived viremia begins to fail, allowing viral propagation within the target cells in circulation. This should be why the decrease in CD4 count at this juncture goes all the way down as opposed to what happens at primary infection. In theory, the onset of AIDS may be caused by the general failure of the immune system including failure of the specific immune response against HIV and probably not the depletion of CD4 count. Death

occurs with very high viremia, comparable to levels at primary infection while the CD4 count is almost zero. Again this reinforces the fact that this viremia is not related to viral production in circulating CD4 lymphocytes. The fact that levels reached are so high means that the source is a highly productive reserve that cannot be the vanquished primary targets in circulation. This source may the niche and other productive secondary tissues.

The niche is not a constant in physiology. It is a concept of microscopic compartments formed in the bone marrow according to the hematopoietic demand, and as such, the number increases with the increase and persistence of this demand. When these compartments are formed, they uptake and concentrate the requirements for cell division and differentiation, much the same way construction material is assembled at a site. Because the niche comprises of the highest concentration of primary target cells for HIV where they are restricted to complete development, infection means the cells will reach viral production while still in the niche, and therefore discharge their viral load within the niche to cause infection of fresh cells forming there. This becomes a cycle that can be called progenitor to progenitor transfer which in theory is critical for maintenance of the reserve infection responsible for chronic HIV infection. Furthermore, the cells which discharge within the niche would die, fueling the demand for more production of CD4 progenitors in a vicious cycle sort of scenario. Evidently, the niche is protected from the rest of circulation by physiological isolation, giving the HIV reserve infection there a free hand to operate without interference even with the onset of specific immune response at sero-conversion.

Theoretically high viremia is required for seeding of the niche. It might also be assisted by the rapid progenitor production induced by sudden decrease in CD4 count; probably both play complimentary and critical roles. It means for productive infection to confer progenitor to progenitor transmission in the niche, a certain minimum quantity of virus must penetrate into the niche; otherwise the infected progenitors could leave the niche before viral maturity, and upon maturity, the produced virus must find the next progenitor in the lineage ready to be infected. Indeed this has been partially demonstrated by the infected CD4 trafficking that occurs at the peak of primary infection. Seeding of the niche may occur only when there is opportunity for CD4 trafficking and, as such cannot occur when there is sufficient peripheral viral control such as provided by a competent immune response and HAART. Seeding of the new niche units as they form appears to occur opportunistically during the course of retroviral disease in situations that allow escalation of viral loads such as immune-depression. Re-infection in HIV patients also probably occurs in circumstances that provide this or similar window. Variability in the trends and prognosis of individual HIV patients may be explained in the context of the niche. If individual delays in obtaining sero-conversion at primary infection, they will invariably have more niche units infected as they form, than in one who reaches ser-conversion sooner, hence the former would have a poorer prognosis. Similarly, early institution of HAART has been linked to much higher CD4 recovery and better prognosis probably because it prevents widespread attack of more niche units as they form. Patients at the very near death end put on HAART almost never recover their CD4 levels probably because their niche capacity is already exhausted and most of what they have already infected.

Primary HIV infection is similar to flu or mononucleosis and resolves spontaneously without any specific treatment.[32-34] there is no direct mortality associated with primary HIV, and even the symptoms of primary infection are mostly attributable to host immune response rather than any direct viral action. On the other hand primary infection by measles, flu, polio, chicken pox, and rabies are much severer and can lead to potentially fatal complications such structural epithelial damage (hence pneumonia) and functional neurological damage, and direct death. The explanation would be, because HIV infection is highly disseminated, specific, and targets cells that have no essential structural function, it is able to avoid intense local inflammatory reaction and structural damage that can lead to direct killing of the host organism at an early stage.

2.3. Immunization Against HIV

The immune system mounts a specific response which, although competent, [7] does not eliminate the infection because of constant resupply from sequestered reserve in the bone marrow. There are no known survivors to HIV who have been shown to be immune at the moment. Even those with active infection remain vulnerable to re-infection[35] in spite of the running specific response being present. This may mean that the presence of active infection may hinder the preventive immune response to new virus or the response is not sufficient to prevent seeding of new infection, old infection notwithstanding. The same mechanism that allows re-infection in HIV patients could allow infection in vaccinated individuals. The emphasis is on the fact that the immune response mounted is the best the system could ever achieve as it is a result of sufficient antigenic exposure by widely disseminated primary infection, and that it is actually competent to eliminate the infection in the absence of sequestered reserve in the bone marrow. It follows then that it may be the immune down-regulation and immune suppression may play a role in HIV re-infection.

There is all unlikelihood of obtaining a vaccine that can elicit a better specific response than the virus itself. Sustained activation of the specific response due to constant exposure to virus can take a toll on the body increasing nutritional demand and straining vital resources that are needed to deal with other issues. With time the active immune response is down-regulated by gene-mediated immune-modulation [36, 37] thus further reducing the effectiveness of the specific response.

HIV vaccination so far has only achieved partial immunity, [38, 39] as has been demonstrated by many attempts.

Dr. Frank Plummer's[40] observations with commercial sex workers (CSWs) in Nairobi can be probably be explained in this context. The phenomenon is could be partial immunity and it occurs in other infections as well, including malaria. When an individual is exposed to sub-infective dosage of a contagion, they can develop a specific immune response before the contagion is able to disseminate into primary infection and thus attain immunity to that infection or any subsequent exposures as long as the specific response remains active. This immunity is only partial as the exposure is not sufficient, and therefore cannot be maintained for ever. The subject may become susceptible once the response is deactivated. In the case of CSWs in Nairobi, these were all engaged in high risk behavior of having unprotected sex with multiple high risk partners every day, allowing them regular exposures, as in 'booster' doses. It is probable that these individuals may have had sub-infective initial exposure and subsequent regular doses resulted in maintenance of specific response hence partial immunity. A future successful HIV vaccine would be the one capable of eliciting a memory that would cause an exposure response that is consistent and strong enough to prevent formation of bone marrow reserve infection.

3. Justification

HIV/AIDS is a global pandemic affecting millions of people with far reaching health and economic challenges.[41] As such, a practical cure attempt must be applicable to a vast majority of all cases by being simple to administer, economically achievable, of acceptable health risk profile and not socially controversial.

3.1. Case Study

A 27 year old black man and a 40 year old black woman, both diagnosed with HIV infection and had been treated with HAART (600mg of efavirenz once daily, 150 mg of lamivudine twice daily and 30 mg of stavudine twice daily) for the previous 6 years for the female and ten years for the male, were treated using methotraxate in a methodology that was aimed at eliminating bone marrow infection and hastening the removal of secondary HIV reserve. Methotraxate was titrated over 18 hours to achieve bone marrow saturation and the end point was determined by the onset of systemic effects associated with methotraxate. This titration was repeated after one month. At the time of commencement of treatment, HIV viral load, full blood count and platelets, CD4 and CD8 counts and Liver function were done. Viral load was determined using quantitative HIV PCR (roche) on Cobas Ampliprep/Cobas Taqman HIV-1Test v2 analysis platform available from private certified pathologists in Nairobi, Kenya. Since the blood picture obtained for these patients did not show any deficiencies, only mineral and vitamin supplements were given to maintain the immune system activity. Subsequent monitoring continued periodically every month or so at the same facility for the last eight months.

3.2. Case Data

1. Reversal of viremia on methotraxate without HAART; this occurred at the beginning of this treatment and is remarkable in that it shows that the theory of bone marrow sequestration and reserve infection is indeed true, as methotraxate has no direct anti-HIV activity yet it was able to effect this reversal.

2. Healing of patients: the female patient who had very scanty scalp hair with massive dandruff and fungus (was using wigs) has shown signs of physical rejuvenation after the procedure with complete skin renewal, growth of healthy follicular hair and resolution of numerous scratch injuries present before the treatment, weight gain, absence of numerous opportunistic infections involving the respiratory tract and the digestive system characteristically dominant before treatment, and general glow of life with improved sleep. The male patient also recorded similar healing.

3. The patients achieved undetectable viral loads without HAART after 8 weeks for the male and ten weeks for the female and have remained without any issues to the date of this report.

4. Failure of viral rebound for eight months after stoppage of HAART shows that the patients are progressing well on the path to eventually eliminate all vestiges of the virus from the body. It is a demonstration of cure.

5. Continuous monitoring is ongoing.

At the time of this assessment, the patients were found to be clinically cured of HIV according to the criteria discussed below. The patients did not experience adverse side effects that would necessitate discontinuation of the treatment. However, it was noted that some sequels of the treatment needed special attention even though this could be achieved without taking the patient to admission.

3.3. Conclusion Criteria

HIV cure can be described as failure of peripheral viral load resurgence and peripheral viral clearance on stoppage of highly active antiretroviral therapy (HAART) in a treatment experienced individual with remarkable viral load control.[1, 2] In these individuals, viral load is expected to begin rising within days following stoppage of HAART.[1, 3,4]This increase should be sustained to full carrying capacity so long as HAART is not reinstated. It should be expected that the final viral load levels in such a patient should be in the region of several million HIV-1 RNA copies per milliliter of blood as described by evidence in a previous cure report.[1] In other circumstances, the viral load progress would depend on the stage of the patient's retroviral disease as can be deduced from the general trend of viral loads in retroviral disease (figure 1) to be in the range of between 350,000 and 700,000 copies per mL.

The immediate increase would be caused by the virus reverse disseminating from the bone marrow niche, and not

the virus derived from circulating CD4 T-lymphocytes or other known secondary reservoirs such as gastric associated lymphoid tissue (GALT) and long live lymphocytes of CD4RO phenotype,[4, 5] as these are suppressed in HAART and are unable to cause such a rapid initial rise. These secondary reservoirs, as well as circulating CD4 lymphocytes are protected from viral activity especially in the presence of a competent immune response and prior viral suppression by HAART, and would require much longer time of reseeding and resumption of active viral production. Indeed rebound viremia has been characterized to be from other active sources previously unknown,[5] and now elucidated to be the bone marrow[6] particularly probably from the CD4 lymphocytopoietic niche, which is the unproved concept of the production unit of CD4-lymphocytes in the bone marrow. Although the secondary tissues are protected in immune-competent individuals on HAART, this protection is not absolute and some baseline viral activity does occur. As such, for these tissues to become productive enough to cause rebound viremia, they need to be sufficiently re-infected, and this eventually occurs if there is persistent viremia from another source; the niche. Peripheral viral production appears to play insignificant role in disease progression in immune-competent patients on HAART, allowing them to live apparent normal lives. Nevertheless, the disease progresses within the bone marrow niche with significant effect on life expectancy.

Once the reserve infection has been eliminated, reseeding of the bone marrow niche from plasma viremia is not expected as it has been shown that rebound viremia in subsequent structured treatment interruption (STI) studies consists of viral quasi-species present before initiation of therapy and not the quasi-species present in circulation.[5]It means rebound virus in peripheral circulation does not usually reenter the bone marrow niche to contribute to the reserve infection therein. If this were the case, the circulating quasi-species would appear in the subsequent rebound viremia. This may be due to the fact that seeding of the bone marrow niche requires sufficiently high viremia, may be to the tune seen at the peak of primary infection,7 a situation that cannot be reached in a patient on HAART and who has a competent immune response. Indeed, the use of post-exposure prophylaxis (PEP) in HIV prevention8 relies upon prevention of peaking of viremia to levels that can cause bone marrow seeding and thus confer chronic infection, thereby allowing the immune system to eliminate the virus before the sequestration has occurred.6 After sero-conversion, and on commencement of HAART, plasma viremia is controlled by both the immune response and drugs. The immune response protects the primary target cells (CD4 T-lymphocytes) from productive viral infection by antibody action and killing of infected cells before they can discharge new virus. This also means that the CD4 lymphocytes cannot cause dissemination of the virus by trafficking into the niche. The seeding of the niche probably occurs before the onset of the specific anti-HIV immune response, and is dependent on cellular viral trafficking by infected CD4 T-lymphocytes into the niche.8 At this time, susceptible stages of the CD4 progenitor cells can be sufficiently productively infected to cross infect newly differentiating counterparts, which will in turn infect more cells as they enter the susceptible stage in the lineage. At low viremia, the numbers of infected circulating CD4 cells is too low to cause such trafficking, and the presence of specific immune response means most of those infected will be killed before they can discharge their load into the niche. It is important to appreciate that niche seeding and reseeding could theoretically occur at any time in the course of retroviral disease, that is, if circumstances that allow sufficiently high levels of viremia to be achieved, as is the case for re-infection in HIV patients. This may explain why the quasi-species in the niche remain unchanged throughout the course of HAART while those in peripheral circulation undergo various mutations, perhaps because of their much lower efficiency of productive infection due to involvement of non-primary target cells and disruptions by the immune system. On the other hand, it may not be possible to eradicate infection in the niche probably because immune action is absent or insufficient there, making the niche the ultimate reservoir for HIV.

Maintenance of viremia in the bone marrow niche requires effective progenitor to progenitor transmission and if this relay is disrupted, restarting it would require fresh seeding. Once the niche is disrupted , in a short while, as evident in a previous cure report,[1] the secondary tissues will be unable to maintain any more viral production as they will die due to viral damage, or will be removed by immune action. Other cells bearing viral infection within the bone marrow such as macrophages and megakaryocytes cannot cause reseeding of CD4 progenitor populations because they may not even be part of the niche in the first place, and their viral production may be too low to provide sufficient virus that can cause productive infection in the progenitors before they mature and leave the niche. As such, any infected progenitors will enter circulation before they can infect others in the lineage within the niche, and will be subsequently killed in circulation by immune action or viral damage before they can reenter the niche to discharge their payloads. Infection of these other cells in the bone marrow also appears to be opportunistically dependent on viral production from the CD4 progenitors and cannot last long on their own. Rather like “a burning stone in the pyre.”

Chronic HIV infection is due to infection of the bone marrow CD4-lymphocytopoietic niche and elimination of this infection alone is required to achieve cure, provided that the re-infection of the niche is ruled out. The period required to prove HIV cure can be roughly predicted to be within several weeks of stoppage of antiretroviral therapy,1, 2, 3 and, more accurately, it is the period required to obtain reversal and clearance of viremia upon withdrawal of HAART in an immune-competent individual with chronic HIV infection and remarkable peripheral control of viremia. This period can be determined by competent methodology

involving assay of various tissues for HIV infection in a research undertaking.

If an agent that can eliminate viral activity in the bone marrow niche is used, reversal of viral load resurgence upon stoppage of antiretroviral therapy demonstrates that viral reserve in the bone marrow niche is no longer productive. Reversal of viremia without HAART, even when peripheral viremia is not yet cleared, is enough to show that the tide has turned on chronic HIV infection, and that this could lead to complete elimination provided the reseeding of the niche is not achieved.

To achieve cure, elimination of bone marrow reserve infection must be followed by elimination of secondary reserve as well as prevention of re-infection of the niche during this period.

3.4. Informed Consent for Patients

This study did not use any experimental substances or procedures that would require approval from an ethics board. However, to participate in this study, the patients were fully informed about it and signed informed consent forms copies of which are available with the Editor in Chief.

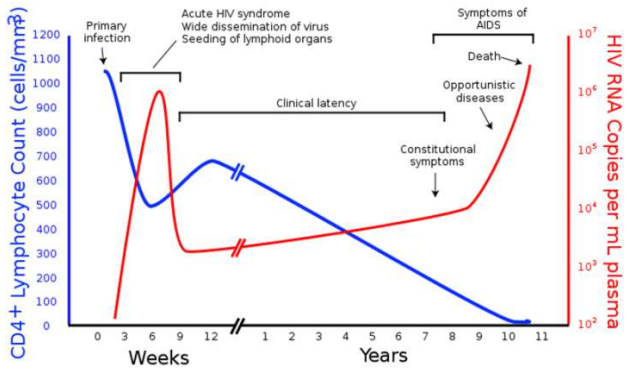

Figure 1. *Illustration of the general trends of viral loads and CD4 T-lymphocyte counts during the course of the HIV retroviral disease*

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Declaration of Interest

The author declares no financial interests in this publication.

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