Has a time to talk about cure of AIDS arrived?

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Abstract

Background :Cure can have different meanings in the context of epidemiology , clinical care and programmatic evaluation as in RNTCP(Revised National TB Control Program).It could range from 'remission(cancer model) to 'eradication' (Infectious diseases model) Antiretroviral drugs have reached the limit of their effectiveness. The cost of providing universal access has become unsustainable, and accumulating evidence underscores the detrimental effects of persistent HIV infection even while plasma viral load is low and CD4 cell count is high .There could be host of factors attributed for this scenario ---- life long treatment involving the cost of therapy and requiring a very high level of adherence unpleasant side effects , including risk for CVD and cancer increasing with age , ultimately HAART failing, leading to resistance, and absence of a therapeutic/preventive vaccine in near future. Naturally ever than before, much need has been felt for the quest for a 'cure'. Achieving either a functional cure (long-term control of HIV in the absence of HAART) or a sterilizing cure (elimination of all HIV-infected cells) remains a major challenge

Two men—both dubbed "the Berlin Patient # 1 & # 2"— who will be remembered as 'harbingers ' in the discovery for a cure have changed the course of history , particularly with the publication of the Berlin patient # 2 case report (2009) with AML BMT from a donor who carried a 32 base pair deletion in the CCR5 gene , has infused new hope in researchers for at least a 'functional cure ' .

Ultrasensitive tests reveal very low levels of plasma HIV RNA (as little as 1 copy/mL) in most people with "undetectable" viral load. Replication-competent HIV can still be isolated from resting CD4 T-cells from people with the longest duration of HAART use—now around 15 years—and viral rebound almost always occurs soon after treatment interruption.HIV can persist inspite of continuing ART as it hides in reservoirs , some of them becoming latent ones , that are not sensitive to current therapies . The most significant barrier to cure is the establishment of a latent or 'silent' infection in resting CD4+ T cells and the persistence of HIV in a latent form in different cellular and anatomical reservoirs Mathematical modeling suggests that it would require 70 years ot treatment with HAART to eradicate latent reservoirs..

Researchers are exploring many approaches for eradicating HIV or achieving a functional cure,which are --- Starting ART very early before viral reservoirs are fully established , Intensifying antiretroviral therapy to stop residual HIV replication

Activating resting T-cells to purge or flush out latent virus , Maintaining latency to keep proviral DNA permanently silenced ,

Eliminating or disabling HIV-containing resting cells , Protecting uninfected cells against viral entry , Strengthening the immune system's response to HIV .

Aim & Objectives : This presentation focuses on the key scientific and clinical variables that we need to understand in order to significantly expand the breadth and scope of the various approaches aimed at finding a cure for HIV. In addition we will focus on limitations of long term HIV Therapy , obstacles coming in finding a 'cure' and explores for eradicating HIV or achieving a 'functional cure '.

Methods/Study Design :
Data Source : The scientific literature, and eligible materials were surveyed related to 'cure/eradication of AIDS
Data Selection: Building on this conceptual framework, the related observational studies and modeliling works,who met the selection criteria of being related to ' cure of AIDS'
Data Extraction : Reports were screened and information from eligible studies was abstracted independently and synthesized.
Design : A descriptive study on the issues of 'cure from AIDS' comprising several randomized and non randomized studies

Results/Findings, : Several randomized clinical trials have demonstrated that treatment intensification with additional antiretrovirals has little impact on latent reservoirs. Some potential other approaches that may reduce the latent reservoir include very early initiation of HAART and the use of agents that could reverse latent infection. Drugs such as histone deacetylase inhibitors, currently used and licensed for the treatment of some cancers; methylation inhibitors; cytokines such as IL-7 or activators of nuclear factor kappa B (NF-κB) such as prostratin, show promising activity in reversing latency in vitro when used either alone or in combination. Alternate strategies include using gene therapy to modify expression of CCR5 and therefore make cells resistant to HIV. One multicenter, open-label, non-randomized trial , called 'New Era study' to evaluate treatment with multi-drug class (MDC) HAART and its impact on the decay rate of latently infected CD4+ T cells has been going on in Germany with primary objective of reducing proviral DNA in PBMC (peripheral blood mononuclear cells)and achieving HIV eradication.The 'EraMune trials are evaluating whether an intensified ART regimen with either Interleukin-7 or a therapeutic vaccine can eliminate HIV from the body.Another investigational approach to curing HIV is modifying CD4 T-cells to make them resistant to HIV entry, by removing CCR5 by a zinc finger nuclease.

Study Limitations; There are multiple barriers to the eradication of HIV infection and despite some recent significant advances in in-vitro models of latency, better animal models and the identification of several compounds that can reverse latency in vitro, there is still a need for more research.

Conclusion : In summary, research to date on HIV eradication and the likely more achievable goal of a functional cure has spotlighted several promising proofs of concept, but none of these approaches are ready for widespread clinical application.. It is likely that multiple combined approaches will be needed to eradicate HIV given that HIV can persist in diverse cell

populations in patients on HAART. A well funded multidisciplinary approach that includes basic virologists, immunologists, clinicians, pharmacologists and the infected community will be needed if we are ever going to meet this challenge.

Keywords : Functional Cure, Eradication, Berlin Patient, HAART, Activated & Latent CD4 Cells , HAART Intensification

Introduction

'Cure' -comes from Latin word 'cura meaning –care, concern , attention'. The current use of word seemingly sprang from the belief that proper and sufficient 'care' was tantamount to 'cure'.
Would that this were so !
The familiar admonition to ***'Cure occasionally,relieve often, console always*** comes from the ancient French aphorism '***Guirer quelquefois, soulager souvent, consoler toujours'***.
This proverb fits superbly into the natural history of HIV/AIDS leading to its 'cure, even it does portray that cure or eradication has a bit of a philosophical content meaning that nobody has a clear concept. We are still in a trial and error phase.
Cure can have different meanings in the context of epidemiology , clinical care and programmatic evaluation as in RNTCP(Revised National TB Control Program in India).It could range from 'remission(cancer model) to 'eradication' (Infectious diseases model)

With the advent of effective comb nation ART in the mid-1990s, some researchers suggested that given enough time, antiretroviral drugs might eventu- ally wipe out all HIV in the body. At the XI International AIDS Conference in Vancouver in 1996, David Ho from the Aaron Diamond AIDS Research Center proposed that a "hit early, hit hard" strategy using a potent combina tion regimen could potentially eradicate virus-infected T-cells—and with them, the virus—within two to three years. Around the same time, however, Robert Siliciano and his team at Johns Hopkins were conducting research that would yield a more sobering finding: In the May 8, 1997, issue of *Nature*, they reported that HIV can hide in a "reservoir" of long-lived resting CD4 T-cells. Because it is not actively replicating, this virus is invisible to the immune system and out of reach of antiretroviral drugs. HIV's genetic blueprint, known as proviral DNA, can lie dormant for years or even decades within a host cell's chromosomes, ready to produce new virus when the cell is activated

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publication of the Berlin patient # 2 case report (2009) with AML BMT(Bone Marrow Therapy) from a donor who carried a 32 base pair deletion in the CCR5 gene , has infused new hope in researchers for at least a 'functional cure ' .Ultrasensitive tests reveal very low levels of plasma HIV RNA (as little as 1 copy/mL) in most people with "undetectable" viral load. Replication-competent HIV can still be isolated from resting CD4 T-cells from people with the longest duration of HAART use—now around 15 years—and viral rebound almost always occurs soon after treatment interruption.HIV can persist inspite of continuing ART as it hides in reservoirs , some of them becoming latent ones , that are not sensitive to current therapies . The most significant barrier to cure is the establishment of a latent or 'silent' infection in resting CD4+ T cells and the persistence of HIV in a latent form in different cellular and anatomical reservoirs Mathematical modeling suggests that it would require 70 years ot treatment with HAART to eradicate latent reservoirs..

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The history of infectious diseases frequently includes people who were resistant to a pathogen. Such a phenomenon helped the Spanish, who had resistance to smallpox, in their conquest of South America, but not the Aztecs or the Incas, who had no resistance to smallpox and were decimated by the virus. Microbial resistance involves adaptive (acquired) immunity (e.g., the HLA subtype) or innate (natural) immunity resulting from the genetic makeup of the host.

With the human immunodeficiency virus (HIV) and its known destruction of the immune sys tem, resistance to infection and disease was not initially expected. However, certain people — long-term survivors — have been infected with HIV for more than 10 years, (and sometimes 30 years) and received no treatment yet remain with- out disease. In addition, some people who have been exposed to HIV on many occasions do not become infected.[3] Both long-term survivors and those who have been exposed to HIV but remain sero negative offer a great opportunity to study the mechanisms of resistance to HIV infection and disease.

HIV enters cells primarily through attachment to the CD4 molecule and subsequent binding to co receptors, of which two chemokine receptors, CCR5 and CXCR4, are the most common. R5 HIV types bind to CCR5; X4 HIV types use CXCR4. People whose cells lack expression of the *CCR5* gene are markedly resistant to HIV infection de- spite multiple exposures to R5 HIV, which is the most prominent virus detected after transmis- sion.This mutation is found in 1 to 3% of the Western population. Among people with HIV who have only one copy of the wild-type *CCR5* gene, progression to disease appears to be slower than among those who have two.[4,5] Obviously, such information is of value in efforts to develop new approaches for therapy

Despite the significant reduction in morbidity and mortality following combination antiretroviral therapy (cART) or HAART (Highly Active Anti retroviral therapy), cART cannot eradicate HIV. Recently, there has been a renewed scientific interest in developing new strategies to eventually find a cure for HIV. There have been several significant advances in our understanding of the major barriers to curing HIV. These barriers include long-lived latently infected cells and residual viral replication, at least in some patients. In addition, anatomical reservoirs including the gastrointestinal tract, lymphoid tissue and the central nervous system

(CNS) may harbour unique long-lived infected cells and penetration of cART may be limited at these sites. The complex mechanisms of how latency is established and maintained in different T-cell subsets and the major cellular reservoirs that persist in patients on cART have recently been extensively reviewed elsewhere .

Objectives

This presentation focuses on the key scientific and clinical variables that we need to understand in order to significantly expand the breadth and scope of the various approaches aimed at finding a cure for HIV. In addition we will focus on limitations of long term HIV Therapy , obstacles coming in finding a 'cure' and explores for eradicating HIV or achieving a 'functional cure '.

Methods /Study Design

Data Source : The scientific literature, and eligible materials were surveyed related to 'cure/eradication of AIDS
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a) Garnering Consensus for Cure :

In the lead-up to the 5th International AIDS Society (IAS) Conference on HIV Pathogenesis, Treatment and Prevention in the summer of 2007, the consensus around the futility of a cure was starting to crack. The approval of two novel classes of antiretroviral drugs—integrase inhibitors and CCR5 antagonists—offered for the first time in years the ability to target HIV at more stages of its life cycle.

The prospect of a cure really came into its own in the summer of 2010. At the XVIII International AIDS Conference in Vienna, Sharon Lewin from Monash University gave an opening lecture highlighting the issue, garnering international media attention.

Preceding the conference, the International AIDS Society sponsored a workshop titled "Towards a Cure: HIV Reservoirs and Strategies to Control Them," which brought together 200 researchers and advocates to discuss the latest advances in the field. A related satellite session at the 2011 IAS Conference in Rome will look at "Controversies in HIV Cure Research."

At the 18th Conference on Retro- viruses and Opportunistic Infections (CROI 2011), researchers presented the first data from a human trial of an experimental gene therapy approach that deletes CCR5 receptors from T-cells in an attempt to halt HIV entry.

At the same meeting the IAS launched an international working group to develop a consensus on the state of HIV reservoir science, define research priorities for tackling persis tent virus, and advocate for increased funding. The group will meet again in Rome and plans to release a formal strategy report at the 2012 International AIDS Conference in Washington, DC.

b) **Need a cure for HIV ?**

Even with the major successes of cART, full life expectancy for patients living with HIV has not been restored. Although some cohort studies have shown near normal life expectancy for a subset of patients ,other studies have shown that life expectancy remains shortened In a prospective study of 3990 HIV-infected individuals in Denmark, the chance of a person with HIV reaching the age of 70 was 50% that of uninfected population controls The incidence of significant morbidity also remains elevated despite successful cART (reviewed in due to complex interactions between drug toxicity persistent inflammation and risk behaviours Antiretroviral drugs have reached the limit of their effectiveness. The cost of providing universal access has become unsustainable, and accumulating evidence underscores the detrimental effects of persistent HIV infection even while plasma viral load is low and CD4 cell count is high .There could be host of factors attributed for this scenario ---- life long treatment involving the cost of therapy and requiring a very high level of adherence unpleasant side effects , including risk for CVD and cancer increasing with age , ultimately HAART failing, leading to resistance, and absence of a therapeutic/preventive vaccine in near future. Naturally ever than before, much need has been felt for the quest for a 'cure'. Achieving either a functional cure (long-term control of HIV in the absence of HAART) or a sterilizing cure (elimination of all HIV-infected cells) due to the lack of financial resources to support lifelong treatment, for everyone in need of treatment, is still a major challenge .

c) Functional or sterilizing cure?

There are two potential strategies for cure. The first is what could be considered an 'infectious diseases model' of cure which would require the elimination of all HIV-infected cells in all compartments and sanctuaries and for patients to have a plasma HIV RNA count of less than 1 copy/ml. This is now commonly referred to as a sterilizing cure. The alternative approach would be to aim for remission or what could be considered a 'cancer model' of cure, in which an individual would have long-term health in the absence of treatment, with low-level viraemia at less than 50 copies/ml. This is commonly referred to as a functional cure

d) Sterilizing cure: elimination of HIV following bone marrow transplantation

The recent case report of a German patient with acute myeloid leukaemia, who received a bone marrow transplant from a donor who carried a 32-base pair deletion in the CCR5 gene, is the only current example of a sterilizing cure . Following transplantation, the patient stopped cART and HIV RNA remained at below 1 copy/ml. In more detailed studies, including multiple biopsies of his gastrointestinal tract, analysis of his cerebrospinal fluid (CSF) and bone marrow and even a brain biopsy, neither HIV DNA or HIV RNA was detected . The patient has now been off cART for over 45 months and HIV is still not detected. Reconstitution of circulating and mucosal CD4+ T cells that did not express CCR5 was observed . CCR5+ macrophages were detected early post transplantation in the gastrointestinal tract but at later time points, all mucosal macrophages expressed the mutant

CCR5 . In addition, the patient's peripheral blood mononuclear cells (PBMCs) were permissive to CXCR4 using laboratory isolates ex vivo, demonstrating that the patients CD4+ T cells were not resistant to HIV. Potential factors leading to the elimination of long-lived reservoirs in this patient could have included the specific chemotherapy administered, total body irradiation or low-grade graft-versus-host disease in addition to eliminating the capacity for any residual replication by removing target cells that express CCR5. Whereas a strategy of using bone marrow transplantation with a CCR5 mutant donor is not a realistic cure for HIV given the toxicity and complexity of the treatment, we need to continue to comprehensively study this patient to fully understand how and why HIV was eliminated.

e) Functional cure: elite controllers

Elite controllers represent a unique group of patients who are able to achieve a consistent and long-term control of viral replication with HIV RNA of less than 50 copies/ml in the absence of cART. In addition, the reservoir is significantly smaller in elite controllers with low concentration of HIV DNA in different subsets of circulating CD4+ T cells in blood as well as in rectal tissue .

There have been multiple studies examining the role of genetics, the virus and the immune response in elite controllers . One of the consistent results from this work is the clear association with HLA class one genes. Recent work has also demonstrated the importance of an effective cytolytic CD8+ T-cell response in blood which has been associated with enhanced activity of the T-box transcription factor t-bet and increased production of IL-21 . Strong HIV-specific CD4+ and CD8+ T-cell responses were also identified in mucosal tissue from elite controllers .The innate immune system may also be important with enhanced activity of myeloid dendritic cells. These data provide supportive evidence that inducing an effective immune response, perhaps via vaccination, may be one strategy to achieve a functional cure.

As some elite controllers do not bear the protective alleles HLA B27 or HLA B57, mechanisms other than enhanced T-cell immunity have also been explored. Several investigators have demonstrated lower replicative capacity of the virus isolated from elite controllers [34-36], and very low level of viral replication soon after infection .There is no evidence currently that activated CD4+ T cells from these patients are resistant to HIV .

Despite apparent 'functional cure' in elite controllers, it is important to remember that low-level viraemia and infected resting CD4+ T-cells are detected. Compared with patients receiving cART, PBMC from elite controllers have similar levels of total DNA, but significantly lower integrated DNA and higher 2-long terminal repeat (2-LTR) levels . Immune activation is higher in elite controllers compared with healthy controls .In contrast to patients on cART with HIV RNA below 50 copies/ml, there is evolution in HIV RNA sequences in elite controllers and in approximately 7% of elite controllers, CD4+ T cells decline over time. Because of the low total number of infected cells and robust HIV-specific immune responses, elite controllers could potentially be the best candidates to test strategies aimed at achieving a sterilizing cure.

Results/Findings

Several randomized clinical trials have demonstrated that treatment intensification with additional antiretrovirals has little impact on latent reservoirs. Some potential other approaches

that may reduce the latent reservoir include very early initiation of HAART and the use of agents that could reverse latent infection. Drugs such as histone deacetylase inhibitors, currently used and licensed for the treatment of some cancers; methylation inhibitors; cytokines such as IL-7 or activators of nuclear factor kappa B (NF-κB) such as prostratin, show promising activity in reversing latency in vitro when used either alone or in combination. Alternate strategies include using gene therapy to modify expression of CCR5 and therefore make cells resistant to HIV. One multicenter, open-label, non-randomized trial , called 'New Era study' to evaluate treatment with multi-drug class (MDC) HAART and its impact on the decay rate of latently infected CD4+ T cells has been going on in Germany with primary objective of reducing proviral DNA in PBMC (peripheral blood mononuclear cells)and achieving HIV eradication.The 'EraMune trials are evaluating whether an intensified ART regimen with either Interleukin-7 or a therapeutic vaccine can eliminate HIV from the body.Another investigational approach to curing HIV is modifying CD4 T-cells to make them resistant to HIV entry, by removing CCR5 by a zinc finger nuclease.

a) Modalities of measuring latently infected cells and the 'reservoir' in vivo

The major reason why HIV cannot be cured is the persistence of HIV in a latent form in different cellular reservoirs. In vivo, HIV latency occurs in resting CD4+ T cells either as preintegration or postintegration latency. Preintegration latency refers to unintegrated HIV DNA that is unstable and will either degrade or will integrate into the host cell genome, usually following cell activation .Postintegration latency refers to the presence of integrated HIV DNA in cells that are not actively producing viral particles .

The major reservoir of cells that harbour postintegration latency in vivo are resting central memory (CD45RA-CCR7+CD27+) and transitional memory (CD45RA-CCR7-CD27+) CD4+ T cells [43,44]. Latent infection can also be established in other long-lived cells including naïve T cells ,bone marrow progenitor cells ,thymocytes and astrocytes Other cells such as monocyte/macrophages can support long-lived low-level productive infection

Together, these persistent infected cells constitute the 'latent reservoir'. Latently infected cells can be detected in both blood and tissue, including the gastrointestinal tract, genital tract and the central nervous system .When activated, latently infected T cells can either release viral particles or became productively infected T cells. In the presence of treatment, further rounds of infection do not occur and there is no viral rebound but when treatment is stopped, viral rebound will occur. There are multiple methods currently used to quantify persistent HIV-infected cells in patients on cART

b) Replication-competent virus: cell associated

The gold standard used to measure the frequency of resting CD4+ T cells carrying latent but replication-competent virus is based on co-culture of highly purified resting CD4+ T cells from the patient together with PBMCs from an HIV-negative donor and is measured as infectious units per million cells (IUPM). The major limitation of using this technique in large multisite clinical trials is the need for large blood volume, often requiring leukapheresis. In addition, the assay is labour-intensive, has a wide coefficient of variation and cannot be performed with tissue biopsies.

c) HIV DNA: unintegrated, integrated and circular

Within infected cells, HIV DNA can exist as linear nonintegrated forms, circular forms and as an

integrated provirus (Fig. 1). In patients receiving effective cART, the majority of HIV DNA is integrated in resting latently infected CD4+ T cells One popular and widely used technique to quantify the number of cells that contain integrated virus is called Alu-LTR PCR .

Quantification of 2-LTR circles that are episomal forms of nonintegrated HIV DNA containing two copies of the LTR is also a useful tool. 2-LTR circles are produced following infection of a cell and have a relatively short half-life. Therefore, detection of 2-LTR circles is generally considered to be a surrogate marker of recent infection, rather than a marker of the number of residual infected cells In a recent small study of six patients who ceased cART, envelope sequences derived from 2-LTR circles from PBMC collected prior to cART cessation were related phylogenetically to envelope sequences from the rebound virus in plasma following cessation of cART In contrast, envelope sequences from PBMC from proviral DNA clustered less frequently with the rebound virus providing further evidence that 2-LTR circles are a surrogate measure of replicating virus

Total HIV DNA quantifies integrated and nonintegrated DNA as well as latent and defective virus. There is a strong correlation between total HIV DNA and integrated HIV DNA in patients on cART and therefore cell-associated HIV DNA is likely to be a good surrogate marker of the total number of latently infected cells In a recent small study, three of seven patients examined had an excess of unintegrated DNA compared with integrated DNA suggesting that total HIV DNA may not be an ideal way to quantify the reservoir in all patients Quantification of total cell-associated HIV DNA is likely to be the most feasible tool to evaluate the frequency of infected cells in large-scale clinical trials and cohorts.

d) HIV RNA: plasma

The quantification of low-level viraemia in patient plasma is now possible using an ultrasensitive PCR-based assay that can measure down to 1 copy/ml .Using this assay, over 80% of patients on cART have detectable viraemia at around 3-5 copies/ml . When HIV RNA is below 50 copies/ml the sequence of plasma virus over time is very stable and shares little homology with the sequence of HIV derived from resting CD4+ T cells or monocytes [63,64]. Further work is needed to understand the source of low-level viraemia and whether this virus contributes to viral rebound.

e) HIV RNA: cell associated

Measurement of cell-associated HIV RNA, includes quantification of extracellular or cell-associated unspliced and multiply spliced RNA (Fig. 1) .In latently infected cells, one would expect no extracellular HIV RNA if ongoing viral replication has been blocked. Indeed, following cART in patients with chronic infection, extracellular HIV RNA rapidly declines, whereas unspliced RNA persists in approximately 70-80% of patients . Multiply spliced RNA is critical for the production of tat, nef and rev, which are all required for efficient production of full-length unspliced RNA In latently infected cells, there is a block in nuclear export of multiply spliced RNA and inefficient production of unspliced RNA Therefore, detection of specific types of multiply spliced RNA differs in patients with productive infection and following suppressive cART .

f) Blood or tissue?

The highest concentration of HIV DNA and cell-associated unspliced HIV RNA in patients on cART is found in tissues such as lymphoid or gastrointestinal tract tissue, as recently

demonstrated in both infected humans and macaques The concentration of HIV DNA and HIV RNA in the gastrointestinal tract is almost 10 times that in blood in patients on suppressive cART In a recent study of anatomical reservoirs in RT-SHIV-infected macaques on cART, lymphoid tissue (including spleen and lymph node) and gastrointestinal tract had the largest pool of infected cells (measured by cell-associated HIV DNA and unspliced RNA), whereas minimal residual infected cells were detected in the CNS or reproductive tract .It is likely that factors that maintain and/or allow the establishment of latency may differ in blood and tissues. Therefore it is critical that for new interventions aimed at eradication, if at all possible, quantification of latently and productively infected cells, should include tissue as well as blood.

g) Barriers to Eradication

Ultrasensitive tests reveal very low levels of plasma HIV RNA (as little as 1 copy/mL) in most people with "unde tectable" viral load. Replication-competent HIV can still be isolated from resting CD4 T-cells from people with the longest duration of combination ART use—now around 15 years—and viral rebound almost always occurs soon after treatment interruption.

i)Sources of Residual Virus

Several research teams have reported that residual HIV in people on ART does not show much evidence of mutation—as would be expected with uncontrolled viral replication—indicating that it likely originates from reservoirs rather than from low-level continuing replication.

But others find evidence that HIV replication may still be occurring despite ART. In the April 2010 issue of *Nature Medicine*, Maria Buzón and colleagues from Spain reported that adding the integrase inhibitor raltegravir (Isentress) to a suppressive ART regimen led to accumula tion of bits of viral DNA known as 2-LTR circles, suggesting that HIV is still copying its genetic material but cannot insert it into host cell chro- mosomes. Using novel assays, Una O'Doherty's group at the University of Pennsylvania also detected uninte grated HIV DNA, suggesting contin ued viral replication.

Most experts now agree that while low-level ongoing replication is likely a factor in some individuals—especially replication in the gut, brain, and other anatomic reservoirs—persistent HIV is largely attributable to virus escaping from a reservoir of latently infected resting immune cells.

ii) Activated and latent CD4 cells

A brief look at the HIV life cycle helps explain why eradicating persistent HIV is such a daunting challenge.

HIV uses surface receptors to enter human cells, primarily the CD4 or "helper" T-cells that coordinate the overall immune response. These cells HIV primarily infects activated CD4 T-cells, or those currently "on duty." Once inside a cell, retroviruses like HIV use their reverse transcriptase enzyme to copy their genetic material from RNA to DNA. Next, the integrase enzyme inserts these new DNA cop-ies into the host cell's chromosomes. Utilizing the cell's own machinery,this proviral DNA blueprint is used to produce proteins which are assembled into new virus particles that burst out of the cell's membrane and go on to infect other cells. Infected activated CD4 T-cells soon exhaust themselves producing new virus and die, or they may be

eliminated by CD8 "killer" T-cells. Initially the body can produce enough replacement cells, but eventually HIV gets the upper hand and the CD4 cell count begins to fall. HIV may also integrate its genetic material into activated CD4 cells that then go into a resting state, and possibly into cells that are already dormant. A reservoir of these latently infected T-cells is established during the earliest stage of HIV disease.

As long as the host cell remains at rest, proviral DNA stays silent; this integrated genetic material allows the virus to "persist as information." Sequestered pro virus in resting cells is hidden from the immune system and invulnerable to currently available antiretroviral drugs. But eventually the host cell may be activated—for example, when it encounters a pathogen it recognizes—which turns on the viral DNA and renews production of infectious virus.

When first produced in the bone marrow, CD4 T-cells are naive, mean ing they have the ability to respond to new threats. Once a T-cell learns to recognize and respond to a specific threat, it becomes antigen-experienced, or "committed."

When an experienced effector T- cell recognizes its target, it proliferates and goes into action. These activated cells burn themselves out quickly,are named for their CD4 receptor, which HIV uses—along with either the CCR5 or CXCR4 coreceptor—to gain entry. Some CD4 cells circulate in the blood, but most reside in lymphoid tissues such as the lymph nodes and lining of the gut. typically within a day or so. Normally the body produces enough T-cells to replace those that are lost, thus main- taining homeostasis, or a steady state.

But a subset of CD4 T-cells lives much longer. After mounting an immune response, some antigen-experi- enced cells—called memory T-cells— go into a resting state. These long-lived memory cells, with a life span of years or decades, act as sentinels, enabling the immune system to recognize and respond more rapidly to threats en- countered in the past.

The absolute number of rest ing memory CD4 T-cells harboring replication-competent virus is small— on average about one in a million resting CD4 cells, or as few as one in ten million in a person on long-term suppressive ART—but this is enough to reignite disease progression if treatment is stopped. There is further specialization within the memory CD4 T-cell popu lation. Nicolas Chomont from the Vaccine and Gene Therapy Institute (VGTI) and others have shown that central memory T-cells (the longest- lived type) and transitional memory cells are the main reservoirs of latent HIV. Central memory cells eventually die off, but proviral DNA in transitional memory cells may be copied into daughter cells as they undergo homeo- static proliferation (ongoing division to maintain a steady level).

The VGTI team found that mostly central memory T-cells make up the latent HIV reservoir in people who start ART early and respond well with large CD4 cell gains, while people with poor CD4 cell recovery have more latently infected transitional memory cells. Since these two cell types enable HIV persistence in different ways, the researchers concluded that complete viral eradication will require a combi- nation approach.

iii) Other Reservoirs :

Researchers have long debated whether hidden HIV resides in other cellular reservoirs besides resting CD4 T-cells. Proposed candidates include naive CD4 T-cells, monocytes and

macrophages, dendritic cells, and stem cells in the bone marrow. Viral dynamics, or how HIV levels change after starting treatment, gives clues about the nature of these reservoirs. In a keynote lecture at CROI 2009, Siliciano argued that decay patterns and gene sequencing indicate that residual virus is coming from a second, unknown cellular reservoir in addition to memory CD4 T-cells.

Macrophages and their precursors, monocytes, carry receptors that HIV can use for entry. Siliciano speculated that his proposed second reservoir might be a progenitor cell, or stem cell, further back in the monocyte/ macrophage line.

In the April 2010 issue of *Nature Medicine*, Christoph Carter from the University of Michigan at Ann Arbor and colleagues reported that latent HIV can hide in CD34 hematopoietic stem cells, which give rise to all types of blood cells. When these stem cells were forced to differentiate in the labo ratory, proviral DNA was activated and began producing new virus.

In addition to cellular reservoirs, HIV also hides in areas of the body that act as "sanctuary sites." Within days after infection the virus estab- lishes itself in anatomic reservoirs such as the central nervous system and the gut. In fact, gut-associated lymphoid tissue (GALT) in the lining of the intestines is the largest source of vulnerable CD4 T-cells

In the brain, HIV primarily targets specialized macrophages called microglia; it does most of its damage to brain tissue by triggering inflamma- tion. At the IAS Reservoirs workshop, Melissa Churchill from Monash University reported that autopsies of brains from people with HIV revealed proviral DNA in astrocytes (a type of brain support cell), with more pronounced infection in people with HIV-associated dementia.

iv) Viral latency

How does HIV manage to remain latent in resting T-cells? This process is a topic of intensive investigation, as it offers clues about potential strate gies for flushing the virus out of these cells—a key step in eradication.

The study of how genes are turned on or off is known as epigenetics. Every cell contains the complete human

genome in its chromosomes, but a variety of mechanisms regulate which specific genes are used to manufacture proteins. "The fingernail gene in a tooth cell is turned off forever," eradication researcher David Margolis from the University of North Carolina explained at a February 2010 forum sponsored by the AIDS Policy Project, an advocacy group promoting cure- related research. Multiple mechanisms have been implicated in epigenetic silencing, or turning off proviral DNA in resting CD4 T-cells, as well as reversal of this process when the cell is activated.

HIV has promoter and enhancer elements at one end of its proviral DNA that regulate viral transcription . In a latent state, these regulatory elements are hidden. When a resting cell is activated, the proviral HIV blueprint transcribes just a few genes at first, producing a viral protein called Tat; once a critical amount of Tat is made, replication can accelerate.

Host cell chemical signals help to either maintain HIV in a latent state or cause it to awaken and begin producing new virus. Some of the same factors that trigger human gene transcription do the same for proviral DNA, including nuclear factor kappa- B (NF-kB), nuclear factor of

activated T-cells (NF-AT), and positive transcrip tion elongation factor b (P-TEF). Tat works by recruiting these factors to the HIV promoter element, leading to activation of proviral genes.
In a cell's nucleus, DNA is coiled around structures called histones, al lowing long chains of genetic instruc tions—about two meters in humans— to fit into a tiny space. A unit of DNA wrapped around a histone is called a nucleosome, multiple nucleosomes plus accessory proteins make up chromatin, and chromatin is packaged into chromosomes.
Acetylation, methylation, and phosphorylation are chemical changes that determine whether chromatin is condensed and unusable, or expanded so it can be used to build new proteins.
Histone deacetylases (HDACs) are enzymes that keep DNA tightly bound to histones and therefore inaccessible. HDACs play a key role in maintaining proviral latency; drugs known as HDAC inhibitors reverse this process, enabling expression of proviral DNA and production of new virus.
Activating latent cells to flush out HIV and preventing cell activation to keep the virus permanently silenced are both potential approaches to a cure. But much remains to be learned about HIV reservoirs, how the virus establishes latency and reawakens, and how human host factors influence these processes.

h) Current and future strategies aimed at eradicating HIV: advantages, disadvantages :
Researchers are exploring many approaches for eradicating HIV or achieving a functional cure, most of which can be categorized into a several broad areas:

• Starting ART very early before viral reservoirs are fully established

• Intensifying antiretroviral therapy to stop residual HIV replication

• Activating resting T-cells to purge or flush out latent virus

• Maintaining latency to keep proviral DNA permanently silenced

• Eliminating or disabling HIV-con- taining resting cells

• Protecting uninfected cells against viral entry

• Strengthening the immune system's response to HIV

i) Treatment intensification
There have been a number of studies that have looked at the effect of treatment intensification on residual virus, in patients receiving cART. These studies have included the addition of agents, such as enfuvirtide, additional protease inhibitors (ritonavir-boosted atazanavir or lopinavir) or raltegravir, to an already suppressive regimen Disappointingly, none of these studies have demonstrated any decline in low-level viraemia, IUPM or cell-associated HIV DNA. In addition, two small, nonrandomized studies showed no significant decline in cell-associated HIV DNA or unspliced RNA in the gastrointestinal tract following intensification with raltegravir (n = 7) and no change in HIV RNA in the CSF following intensification with maraviroc, lopinavir/ritonavir or enfurvitide (n = 10)
One randomized study using raltegravir intensification for 48 weeks in 69 patients showed no change in persistent low-level plasma HIV RNA or cell-associated DNA after 24 weeks but

demonstrated clear evidence of residual viral replication in at least one-third of patients as seen by an early and rapid increase in 2-LTR circles Taken together these studies suggest that the addition of the potent antiretroviral raltegravir alone to suppressive cART has minimal effect on persisting low-level viraemia and HIV DNA in either blood or tissue and approaches other than intensification will be needed

Antiretroviral agents from different classes may have unique activity on establishing, maintaining or eliminating latently infected cells. A recent small, nonrandomized intensification study with maraviroc, a CCR5 antagonist, demonstrated some intriguing findings In this Spanish study, maraviroc was added for 48 weeks to a suppressive cART regimen in 10 patients. They observed an increase in HIV RNA (measured by the single copy assay) and 2-LTR circles in association with a significant decrease in the IUPM. This is the only intervention to date that has shown a decrease in the number of latently infected cells (measured by IUPM) using intensification in patients on suppressive cART. The mechanism for how maraviroc may be working is unclear; however, investigation of tissue reservoirs, specifically gastrointestinal tract and lymphoid tissue and repeating this study in a larger randomized design will be important.

One interesting study called 'New Era study ,is being conducted in Germany for Eradication of HIV which is going to have serious impact on prospect of eradication of HIV as it has set certain endpoints /parameters , which is going to decide the future course-- how a cure/eradication and a failure would really be defined .It **is a** multicenter, open-label, non-randomized trial to evaluate treatment with multi-drug class (MDC) HAART and its impact on the decay rate of latently infected CD4+ T cells with primary objective of reducing proviral DNA in PBMC (peripheral blood mononuclear cells) and achieving HIV eradication. With study group comprising i) **PHI (**Primary HIV Infection**) group** N=20 patients with primary HIV-infection .

ii) CHI (Chronic HIV Infection**) group** N=20 patients with chronic HIV-infection and plasma VL < 50 copies/ml for≥ 3 years on PI -based HAART and defined **Study intervention - PHI: 2 NRTI + 1PI + MVC+ RAL, CHR: 2 NRTI + 1PI**…ongoing + add on **MVC+ RAL(**MVC - Maraviroc =Celsentri and RAL –Raltegravir will be delivered as study drugs **)Treatment time:**7 years or until endpoint (eradication or virological failure) **Study visits** CHR: Months -6, -3, BL, M1, 3, 6-monthlly thereafter PHI: Screening/BL, M1, 3, 6-monthlly thereafter.

Other treatments or immunomodulators which may have a potential impact on viral reservoirs will not be discouraged during the course of the study. And the treatment regimen can be modified based on current knowledge.

Study endpoints:

Eradication : Standard VL assay: Plasma VL < 50 cop./ml for≥5 years

AND

Single-copy assay (SCA): Undetectable plasma VL for≥2 years

(VL <1 cop./ml)

AND

Undetectable proviral DNA in PBMC for≥ 2 years

Patients fulfilling the criteria for eradication and willing to stop HAART will be monitored for proviral DNA for a follow-up period of 12 months.

Virological failure :
For CHI: Confirmed virological rebound in plasma viral load to levels >1000 cop./ml
For PHI: Confirmed plasma viral load (>50 cop./ml) 6 months after HAART initiation
OR Confirmed virological rebound in plasma viral load to levels >1000 cop./ml

ii) Early treatment (Hit early hit hard')
Early treatment may be a potential strategy to reduce or even control the number of persistent latently infected cells. Several groups have demonstrated that the number of infected cells, as measured by both cell-associated HIV DNA and HIV unspliced RNA, decreases to a significantly lower level if cART is initiated during acute rather than chronic infection.

In a recent longitudinal study of patients who initiated cART during very early acute infection and stayed on cART for a prolonged period, in five of 32 (16%) patients following cessation of cART, HIV RNA was maintained at below 50 copies/ml for a median of 77 months off cART The use of very early cART may have had a significant impact on the number of infected cells as measured by total HIV DNA However, the findings from this study were in contrast to many other studies of viral rebound in nearly all patients following cessation of cART, even when initiated during acute infection The role of very early treatment initiation in limiting seeding of the HIV reservoir, as well as preserving immune responses capable of controlling HIV replication, requires further investigation.

ii) Elimination of latently infected T cells via induction of virus production
One strategy to eliminate latently infected cells is to induce virus production from latently infected cells. Further rounds of infection would be blocked by cART and the productively infected cell would die. This strategy is only viable if active viral replication is completely inhibited on cART.
iv) Janus kinase-signal transducers and activators of transcription (JAK-STAT) pathway
IL-7 IL-7 is a cytokine that can effectively induce productive infection from latently infected resting CD4+ T cells in vitro via activation of the JAK-STAT pathway [88]. IL-7 has also recently been shown to be well tolerated in patients with HIV infection and leads to the expansion of naïve and memory CD4+ and CD8+ T cells In these studies, a clear but transient increase in HIV RNA was observed, despite all patients receiving cART. The virus detected in plasma following IL-7 was similar phylogenetically to virus prior to IL-7 in both plasma and CD4+ T cells. One concern with IL-7 is that this cytokine may potentially expand not only uninfected cells but also latently infected cells by inducing proliferation of all cells, specifically transitional memory T cells IL-7 is currently undergoing clinical trials (ERAMUNE, www.clinicaltrials.gov), as a strategy to reduce the size of the latent reservoir, and results of this trial are awaited with high interest.

v) Activation of NF-kB: prostratin
There are alternative compounds, such as prostratin, that can promote T-cell activation and HIV transcription in vitro [92]. The large diversity of latently infected T-cell subsets may differ in their capacity to proliferate, and/or uptake of these drugs. However, prostratin has not yet been assessed for safety and toxicity in humans and therefore is unlikely to enter clinical trials in the near future.

vi) Enhance histone acetylation: histone deacetylase inhibitors

Many in-vitro studies have demonstrated that latency can be reversed, that is viral production can be activated by promoting histone acetylation Histone deacetylase inhibitors (HDACis) are drugs that can modify gene expression by changing the acetylation state of histones, leading to enhanced transcription from multiple genes including from the HIV LTR. In cancer cells, HDACis induce cell death and cell cycle arrest of rapidly dividing malignant cells and many HDACis are now in advanced clinical development for the treatment of different cancers. Following treatment of latently infected cells lines with a number of different HDACis, including valproic acid, MCT1, MCT3 and oxamflatin, we demonstrated that preferential apoptosis also occurred in cells that were producing virus .

Valproic acid, a relatively weak and non-toxic HDACi, showed promising effects in a small pilot study however, further retrospective studies failed to demonstrate any benefit from valproic acid in reducing the number of latently infected resting CD4+ T cells A far more potent HDACi, vorinostat (also called SAHA), is licensed for the treatment of cutaneous T-cell lymphoma, is relatively well tolerated in humans and has significant potency in promoting HIV replication from latently infected cells in vitro

In patients treated for malignancy, the main adverse events from vorinostat were fatigue, diarrhoea and thrombocytopenia which occurred with severity of grade 3-5 in 3-5%]. The median time to onset of an adverse event requiring dose modification of vorinostat was 42 days. Following the administration of panobinostat (LBH 257, Novartis), a pan HDACi similar to vorinostat, a change in gene activity in tumour cells and histone acetylation in circulating PBMC was observed within 2 h of administration and gene activity returned to baseline levels within 72 h Given that the onset of gene activation and suppression is extremely rapid with HDACi, it is possible that only a short course of these drugs may be required for reversing HIV latency and this would significantly reduce the likelihood of toxicities and adverse events.

A theoretical risk of HDACis is that they will induce activation of other retroviruses and/or DNA viruses including cytomegalovirus (CMV), hepatitis B virus (HBV) and JC viruses which has been demonstrated in vitro .However, to date there has been no evidence that the clinical use of HDACi is associated with reactivation of DNA viruses .There has only been a single published case series of three patients in which reactivation of EBV and HBV was reported following administration of an HDACi In this small case series, there was a temporal association with reactivation and administration of the HDACi but it is important to keep in mind that these patients were all patients with advanced cancer and immunosuppression. Therefore it remains unclear if HDACis were indeed the cause of any viral reactivation. The long-term impact of HDACi on enhancing the risk of malignancy and/or reactivation of oncogenes or endogenous retroviruses is also unknown

vii) Inhibit DNA methylation: methylation inhibitors

The methylation inhibitor 5-aza-deoxycytadine (decitabine; Dacogen, MGI Pharma Inc.) is a nucleoside analogue that promotes DNA cytosine methylation and also has a similar effect to HDACi in promoting HIV transcription in vitro, but only in a subset of latently infected resting CD4+ T cells .Decitabine was approved by the FDA in 2006 for the treatment of myelodysplastic syndromes. In vitro, decitabine induced HIV transcription from latently infected cell lines and patient-derived latently infected cells; however, this drug was not active in all latently infected cells and the potency was greatest when used in combination with other drugs such as prostratin and an HDACi. Other compounds that inhibit methylation include histone methyltransferase inhibitors (HMTis). HMTis are in advanced development for the treatment of cancer and further

work is underway to determine if these compounds are also active in latently infected cells.

viii) Combination strategies

Several studies have now demonstrated that the potency of different interventions that modulate HIV gene expression may vary in different latently infected cells, depending on the integration site, the degree of transcriptional interference, chromatin structure and methylation of the LTR in the particular cell [92,99-101]. In addition, a combination of strategies, for example SAHA together with prostratin, appears to have greatest potency in promoting HIV transcription, at least in vitro . Most of the studies that evaluate a combination approach have been performed in latently infected T-cell and monocytic cell lines and it is currently unknown whether this approach will also enhance potency in latently infected primary T cells. In addition, it is currently unknown whether any of these compounds work in other latently infected cells such as astrocytes, naive T cells or stem cells. It is likely that the elimination of the diverse latently infected cells found in vivo will require a combination approach similar to what is currently used in cancer treatment

x) Boosting immunity to HIV: therapeutic vaccination

Many natural history studies of HIV-infected patients have shown a clear relationship between virological control and a robust HIV-specific CD4 and CD8 T-cell response raising the possibility that induction of T-cell immunity via vaccination may potentially generate a functional cure. To date the use of therapeutic vaccination, in patients receiving cART, has not been successful . In one of these studies, treatment interruption resulted in a significantly shorter time to viral rebound following therapeutic vaccination compared with placebo

A recent intriguing study in SIV-infected Chinese rhesus macaques treated with two antiretroviral agents and a live inactivated SIV virus combined with a toll like receptor (TLR) agonist polyICLC applied to the tonsil resulted in significant control of viral replication following cessation of cART]. The animals who received the polyICLC vaccine had significantly elevated titres of neutralizing antibodies compared to the control groups suggesting that the generation of neutralizing antibodies may be important in preventing viral rebound following cessation of Cart

x) Making cells resistant to HIV

Future strategies aimed at making CD4+ T cells resistant to HIV are also currently being investigated. Some approaches that may potentially mimic HIV eradication in the HIV-infected German patient include gene therapy to reduce expression of CCR5. This has successfully been performed in mice through the introduction of a zinc finger nuclease into haematopoietic progenitor cells, which effectively disrupts the gene coding for CCR5 in all daughter cells . This led to a reduction in the expression of CCR5 in a subset of transplanted cells. Following HIV infection of these mice, there was a selective advantage for the CCR5-/- cells which subsequently increased, HIV RNA remained low and CD4+ T cells were preserved in both blood and tissue

A recent phase 2 study demonstrated that infusion of autologous T cells transduced with a zinc finger nuclease that inhibited expression of CCR5 was well tolerated in a small study of six HIV-infected patients on cART An alternative approach is to use RNA-based gene therapy to reduce CCR5 expression, as well as specifically inhibit HIV replication This approach was recently investigated in HIV-infected patients and was shown to be well tolerated and that the transduced genes persisted in a subset of cells for 24 months. Although widespread

use of these therapies is many years away, these results are encouraging for the possible development of a gene therapy-based treatment strategy that may achieve a functional cure.

Study Limitations

There are multiple barriers to the eradication of HIV infection and despite some recent significant advances in in-vitro models of latency, better animal models and the identification of several compounds that can reverse latency in vitro, there is still a need for more research.

a)Research Hurdles

Though they have many promising leads to pursue, researchers face a number of challenges as they search for a cure for HIV. The Berlin Patient's story un derlines one such issue: How will we know if someone has completely and permanently gotten rid of HIV?

State-of-the-art viral load tests can now measure plasma HIV levels down to a single copy per milliliter. But it is much harder to detect latent HIV in resting CD4 T-cells. Given the usual estimate that roughly one in a million resting CD4 cells harbors HIV—and the fact that the vast majority of these cells reside in tissues such as the gut—it takes liters of blood to collect just a few. If treatment reduces the number of virus- containing cells by 100-fold, it may not be possible to find them at all using today's technology.

Theoretically, it is conceivable that some people have eradicated HIV without treatment, and therefore never got tested and never came to the attention of researchers. This seems unlikely, however, given that no one who has been followed from the time of acute infection has been known to clear the virus.

But what about people who ap pear to eliminate HIV using the new therapies under development? The IAS Reservoirs workshop last summer featured a debate about the Berlin case, with some skeptics asking Gero Hütter how he could be sure his patient had no residual HIV anywhere in his body.

As long as the Berlin Patient experiences no disease progression, it may not matter whether he harbors hidden HIV—a long-term functional cure is still a major accomplishment. But determining whether HIV is really gone becomes critical when deciding whether and when to discontinue antiretroviral treatment.

How long should viral load remain undetectable before consider ing ART interruption? What should be the threshold for deciding that HIV has bounced back enough to call an experimental approach a failure? How often should people be tested to be confident they are not experiencing viral "blips"? How often do they need invasive tests such as rectal biopsies or spinal taps? And how long do people have to remain apparently virus-free without ART before declaring that they are indeed cured?

With regard to drug development, investigators are devising novel screen ing methods to test large numbers of compounds, looking for those that might have an effect on the viral life cycle. Pharmaceutical companies, including Bristol-Myers Squibb, Gilead, Merck, and Tibotec/Janssen have ongoing programs to search for candi- dates that might play a role in curing HIV.

According to Hazuda, Merck has already screened tens of thousands of compounds, including HDAC in hibitors, and found several dozen that warrant further testing.

Many compounds under study for HIV have already been tested in animals and humans for other indica tions, and some have been approved by the U.S. Food and Drug Adminis tration (FDA), mostly as cancer che motherapies. One agent that strongly activated latent HIV in a recent lab study and is now entering clinical trials—disulfiram (Antabuse)—is used to manage alcohol abuse, illustrating the benefit of casting a wide net.

The fact that some promising compounds are already approved will likely shorten the period of preclinical research before they can enter clinical trials for people with HIV, but other hurdles lie ahead.

Given the excellent safety and effectiveness of modern antiretroviral drugs, cure candidates have a higher bar to clear. Interfering with chromatin remodeling, transcription factors, and other elements of the gene expression process could have harmful effects on human cells, such as triggering the development of cancer. While the FDA allows use of po- tentially dangerous drugs for life-threat- ening conditions—largely thanks to the work of AIDS activists in the 1980s and 1990s—many people feel HIV infection no longer falls into that category.

The regulatory process and clinical trial system generally do not allow testing potentially harmful therapies in healthy people. While it may be acceptable to give a toxic or oncogenic drug to a person with no other good treatment options, the risk may be too high for HIV positive people who are keeping their virus suppressed on ART and have no signs of disease progression.

b)Funding problems

Along with regulatory issues, another major barrier to HIV cure research is inadequate funding.A recent AIDS Policy Project report estimated that in 2009 the U.S. federal government spent less than 3% of its annual $1.5 billion HIV/ AIDS research budget on work that could lead to a cure

. But it is clear that the amount is dwarfed by spending on HIV vaccine research, which even after 20 years has yet to demonstrate much promise in human clinical trials.,Getting new therapies from bench to bedside requires resources at all levels. But money issues will not disappear once a cure is developed. How can we justify the cost of cure research and implementation, many ask, when millions of people worldwide do not even have access to today's standard-of-care ART?

No one expects that a cure for HIV will be cheap. But even a high-tech approach like gene therapy—if it only needs to be done once or at most a few times—might prove cost effective compared with decades of antiretrovi ral treatment, monitoring, and management of ART-related complications .It has been estimated that treating most HIV positive people in low- and middle-income countries at the old World Health Organization CD4 threshold of 200 cells/mm3 —to say nothing of raising the threshold to 350 or 500 cells/ mm3—could consume half the U.S. foreign aid budget within a decade. A basic antiretroviral regimen typically costs around $20,000 per year in the U.S. (though it runs much less in resource-limited countries that take

advantage of generic drugs and special deals with industry). At that price, even the estimated $200,000 cost of a Berlin Patient-style stem cell transplant pales in comparison with perhaps $1 million for a lifetime of ART.

But if history is any guide, even procedures as intensive and costly as stem cell gene therapy will become less expensive over time as techniques are automated and scaled up. And therapy that is administered once or only a few times might also help overcome the shortage of medical personnel and infrastructure needed to deliver lifelong daily treatment in resource-limited settings.

"A cure will require funding commitments, strong community engage ment, rigorous and innovative scientific endeavor and, above all, further collaborative multidisciplinary science with a better connection between basic and clinical research—in short, all the same ingredients that got us where we are today with global anti- retroviral treatment," Barré-Sinoussi A Nobel Prize winner for HIV , in a New York Times editorial marking the 30th anniversary of the first report of AIDS.

"It's astonishing that people are having intelligent conversations about HIV that includes the word 'cure,'" said Jay Lalezari. "The three-pronged approach is gene therapy to manipulate the host, immune-based therapy to manipulate the immune system, and drug therapy to force HIV out of its hiding place in tissue reservoirs. Whether a cure is going to come from one or some combination of all three, I do think it's possible that in our life- time we will be curing HIV."

c) Moving toward clinical trials to test for eradication :

i) Animal models

Given the absence of a robust animal model of suppressive cART, it is currently unclear whether there is a real need for all interventions to first be trialed in macaques to determine efficacy. To date there have been a limited number of antiretroviral agents that are active or can be administered to SIV-infected macaques allowing durable control of SIV RNA to below 50 copies/ml for a prolonged period of time. We know from viral kinetic models of HIV infection that elimination of productively infected cells likely takes more than 3 years Given the high costs of animal maintenance it is difficult to maintain SIV-infected macaques on SIV cART for longer than 48 weeks. Finally most detailed analyses of factors that modify HIV transcription, for example, activity of HDACi and methylation inhibitors, have been performed in in-vitro models of latent HIV infection in human cells and it is unclear whether this can be translated to SIV infection.

Recent work has, however, demonstrated that infection of rhesus macaques with SIVmac239 that contains the HIV-1 RT (clone HXBc2) and envelope genes (RT-SHIV) treated with tenofovir, emtricitabine and efavirenz appears to be a very promising model of suppressive cART Using an ultrasensitive assay to measure RT-SHIV RNA in plasma, these animals had detectable but low-level viraemia of 2-58 copies/ml consistent with suppressive cART in humans Some recent small animal models also show promise, specifically the blood-liver-thymus (BLT) mouse that can be efficiently infected with R5 HIV and HIV RNA declines in response to antiretroviral drugs

ii) Clinical trials

Clinical trials testing strategies for eradication pose unique challenges that require consideration and further debate. First, measuring the reservoir is complex, can require large volumes of cells, can be invasive and no assays have yet been standardized across multiple laboratories . In addition, it is currently unknown what assay will best predict the likelihood of viral rebound following cessation of cART. Second, most studies to date have been small, nonrandomized studies. This approach is appropriate to test the safety, feasibility and potential efficacy of new strategies; however, there is a need for larger randomized studies in this field. Third, most strategies identified to date, that may have an impact on latent reservoirs, have associated toxicities. Careful consideration of the risk benefit for any of these interventions remains challenging for investigators, patients and associated regulatory authorities. Finally, there remains a need for more multidisciplinary studies, including the use of mathematical models to study the dynamics of reservoirs, taking in account half-life of the subsets of infected cells and their homeostatic proliferation

Although there are multiple ways to quantify residual infected cells in patients on cART (Fig. 1), it is currently unknown whether there is a critical threshold at which viral rebound does not occur or whether all infected cells need to be eliminated to prevent viral rebound. A recent study of a patient treated with suppressive cART during primary infection had extremely low levels of IUPM for prolonged periods of time; however, disappointingly viral rebound still occurred in this patient following cessation of cART Therefore, eventually we will need to consider whether cART interruption is an appropriate clinical end point of these studies given the well documented risks of reactivation of viral replication .Moreover, it is possible that reducing reservoirs to levels below the limit of detection of available assays, may not necessarily predict the likelihood of viral rebound after stopping cART.

Conclusion

In summary, research to date on HIV eradication and the likely more achievable goal of a functional cure has spotlighted several promising proofs of concept, but none of these approaches are ready for widespread clinical application.. It is likely that multiple combined approaches will be needed to eradicate HIV given that HIV can persist in diverse cell populations in patients on HAART. A well funded multidisciplinary approach that includes basic virologists, immunologists, clinicians, pharmacologists and the infected community will be needed if we are ever going to meet this challenge.

There are multiple barriers to the eradication of HIV infection and despite some recent significant advances in in-vitro models of latency, better animal models and the identification of several compounds that can reverse latency in vitro, there is still a need for more research. Promising in-vitro strategies need to be tested in well designed and well tolerated clinical trials to demonstrate proof of concept and to determine whether further investment should be placed in these approaches. It is likely that multiple combined approaches will be needed to eradicate HIV given that HIV can persist in diverse cell populations in patients on cART. A well funded multidisciplinary approach that includes basic virologists, immunologists, clinicians, pharma and

the infected community will be needed if we are ever going to meet this challenge..
As for when this might happen, experts are hesitant to give a timeline, mindful of the inaccuracy of earlier predictions. While a preventive HIV vaccine has taken much longer than government officials predicted in the 1980s, effective ART came about faster than many expected.; Some scientists put the timeframe for a functional cure at around ten years, but most expect complete HIV eradication to take longer. .Probably the three-pronged approach is gene therapy to manipu late the host, immune-based therapy to manipulate the immune system, and drug therapy to force HIV out of its hiding place in tissue reservoirs. Whether a cure is going to come from one or some combination of all three, I do think it's possible that in our life- time we will be curing HIV. I think cure is still the Holy Grail. If we knew that we could *cure* the infection and actually *eliminate* the infection, I think mankind would certainly be better off.

References

1. .Allers, K. and others. Evidence for the cure of HIV infection by CCR5-delta-32/delta-32 stem cell transplantation. Blood 117(10):2791-99. March 10, 2011.
2. .Gandhi, R. and others. Raltegravir intensification of patients on long-term suppressive ART does not increase 2-LTR HIV DNA circles in PBMC: results from ACTG A5244. CROI 2011. Abstract 51. Holt, N. and others. Human hematopoietic stem/pro- genitor cells modified by zinc-finger nucleases targeted to CCR5 control HIV-1 in vivo. Nature Biotechnology 28(8):839-47. August 2010.
3. An DS, Donahue RE, Kamata M, et al. Stable reduction of CCR5 by RNAi through hematopoietic stem cell transplant in non-human primates. Proc Natl Acad Sci U S A 2007;104:13110- 5.
4. Archin, N. and others. Expression of latent HIV induced by the potent HDAC inhibitor suberoylanilide hydroxamic acid. AIDS Research and Human Retroviruses 25(2): 207-12. February 2009.
5. Archin, N. and others. Valproic acid without intensified antiviral therapy has limited impact on persistent HIV infection of resting CD4+ T cells. AIDS 22(10):1131-35. June 19, 2008
6. Berger EA, Murphy PM, Farber JM. Chemokine receptors as HIV-1 coreceptors: roles in viral entry, tropism, and disease. Annu Rev Immunol 1999;17:657-700.
7. Buzón, M. and others. HIV-1 replication and immune dynamics are affected by raltegravir intensification of HAART-suppressed subjects. Nature Medicine 16(4):460-65. April 2010.
8. Chomont, N. and others. HIV reservoir size and persistence are driven by T cell survival and homeostatic prolif eration. Nature Medicine 15(8):893-900. August 2009.
9. Chun, T.W. and others. Rebound of plasma viremia following cessation of antiretroviral therapy despite profoundly low levels of HIV reservoir: implications for eradication. AIDS 24(18): 2803-8. November 27, 2010.
10. Cohen J. Building an HIV-proof immune system. Science 2007;317:612-4.
11. Da Fonseca, S. and others. Purging the HIV-1 reservoir through the disruption of the PD-1 pathway. 18th Conference on Retroviruses and Opportunistic Infections (CROI 2011). Boston. Februar y 27-March 2, 2011. Abstract 268
12. de Roda Husman AM, Koot M, Cornelissen M, et al. Asso- ciation between CCR5 genotype and the clinical course of HIV-1 infection. Ann Intern Med 1997;127:882-90.

13. Deeks SG. Challenges of developing R5 inhibitors in antiret- roviral naive HIV-infected patients. Lancet 2006;367:711-3.
14. Deeks, S. and McCune, J. Can HIV be cured with stem cell therapy? Nature Biotechnology 28(8):807-10. August 2010.
15. DiGiusto, D. and others. RNA-based gene therapy for HIV with lentiviral vector-modified CD34+ cells in patients undergoing transplantation for AIDS-related lymphoma. Science Translational Medicine 2(36):p36ra43. June 16, 2010.
16. Glass WG, McDermott DH, Lim JK, et al. CCR5 deficiency increases risk of symptomatic West Nile virus infection. J Exp Med 2006;203:35-40.
17. Jubault V, Burgard M, Le Corfec E, Costagliola D, Rouzioux C, Viard J-P. High rebound of plasma and cellular HIV load after discontinuation of triple combination therapy. AIDS 1998;12: 2358-9.
18. Lalezari, J. and others. Successful and persistent engraft- ment of ZFN-M-R5-D autologous CD4 T Cells (SB-728-T) in aviremic HIV-infected subjects on HAART. CROI 2011. Abstract 46.
19. Levy JA. HIV and the pathogenesis of AIDS. 3rd ed. Wash- ington, DC: ASM Press, 2007.
20. Levy JA. The importance of the innate immune system in controlling HIV infection and disease. Trends Immunol 2001; 22:312-6.
21. Lewin, S. and Rouzioux, C. HIV cure and eradication: how will we get from the laboratory to effective clinical trials? AIDS 25(7):885-97. April 24, 2011.
22. Lewis, M. and others. Gold drug auranofin restricts the viral reservoir in the monkey AIDS model and induces containment of viral load following ART suspension. AIDS 25(11):1347-56. July 17, 2011.
23. Lisziewicz, J. and others. Control of HIV despite the discontinuation of antiretroviral therapy. New England Journal of Medicine 340(21):1683-84. May 27, 1999.
24. Moulton HM, Nelson MH, Hatlevig SA, Reddy MT, Iversen PL. Cellular uptake of antisense morpholino oligomers conju- gated to arginine-rich peptides. Bioconjug Chem 2004;15: 290-9.
25. Palella FJ Jr, Delaney KM, Moorman AC, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. N Engl J Med 1998;338:853- 60.
26. Prescott WH. History of the conquest of Mexico and the his- tory of the conquest of Peru. New York: Rowan & Littlefield, 2000.
27. Reuse, S. and others. Synergistic activation of HIV-1 expression by deacetylase inhibitors and prostratin: implications for treatment of latent infection. PLoS One 4(6):e6093. June 30, 2009.
28. Richman, D. and others. The challenge of finding a cure for HIV infection. Science 323(5919):1304-7. March 6, 2009.
29. Sánchez-Duffhues, G. and others. Activation of latent HIV-1 expression by protein kinase C agonists: a novel therapeutic approach to eradicate HIV-1 reservoirs. Cur- rent Drug Targets 12(3):348-56, March 1, 2011.
30. Savarino, A. and others. "Shock and kill" effects of class I-selective histone deacetylase inhibitors in combina- tion with the glutathione synthesis inhibitor buthionine sulfoximine in cell line models for HIV-1 quiescence. Retrovirology 6:52. June 2009.
31. Shearer GM, Clerici M. Protective immunity against HIV in- fection: has nature done the experiment for us? Immunol Today 1996;17:21-4.

32. Xing, S. and others. Disulfiram reactivates latent HIV-1 in a Bcl-2-transduced primary CD4+ T cell model without inducing global T cell activation. Journal of Virology 85(12):6060-64. June 2011.
33. Yang, H. and others. Small-molecule screening using a human primary cell model of HIV latency identifies compounds that reverse latency without cellular activa- tion. Journal of Clinical Investigation 119(11):3473-86. November 2, 2009.

Annexure : (A)

The 'BERLIN PATIENTS '

In the history of AIDS, two men—both dubbed "the Berlin Patient"—have paved the way to talk about even for a cure, albeit a 'functional one '.

The first Berlin Patient(# 1) *was a young German man who in 1996 sought care due to flu-like symptoms about three weeks after having unprotected sex. His doctor, Heiko Jessen, started him on ART and hydroxyurea, a cancer drug.. hydroxyurea expert Franco Lori described the case at an AIDS conference in Hamburg in 1997*

After starting combination therapy, the man rapidly reached an "undetectable" viral load according to an older test with a lower limit of 500 copies/ mL. When he stopped his drugs a few months later due to a bout of hepatitis A, his HIV viral load stayed undetectable. About five weeks later, he decided to permanently discontinue therapy and his virus remained suppressed.

This Berlin Patient was the first individual known to have achieved "remission" of HIV, and the case made headlines around the world, including a profile in the New York Times Magazine. Lori's team presented further details at CROI 1999 and in the May 27, 1999, New England Journal of Medicine. By that time, Berlin Patient #1 had been off treatment for about two years, still with no plasma viral rebound. But traces of HIV RNA were detected in his lymph nodes, and replication-competent virus was isolated from a small number of resting CD4 T-cells after Robert Siliciano developed a sensitive test.

Although his HIV was not eradicated, the man's immune system managed to control the virus, demonstrating that a

func tional cure is within the realm of possibility ***(The New England Journal of Medicine Volume 340 Number 21 May 27, 1999)***

The second Berlin Patient (# 2) *came to the world's attention a decade later. An American man living in Germany, he underwent treatment for acute myeloid leukemia at Berlin's Charité Medical University in 2006. At that time, he had been HIV positive for more than ten years and on ART for four years, and had undetectable viral load. But he had a history of high viral load*

and disease progression, so was not a natural elite controller. After initial chemotherapy failed, the next step was a bone marrow transplant. Strong chemotherapy was used to kill off white blood cells, which eliminates the cancer but leaves the patient without a functioning immune system. The man then received a bone marrow transplant containing hematopoietic stem cells; the donated stem cells essentially build a new immune system.

The man's doctor, Gero Hütter—a hematologist with no particular experience in HIV—had read that individuals with the CCR5-delta-32 genetic variation are protected against HIV infection. Against all odds, he found a bone marrow donor who was both a genetic match and carried two copies of the uncommon variation, meaning the donor's cells did not express CCR5 receptors.

Berlin Patient #2 stopped ART the day before his first bone marrow transplant in 2007 and afterward received immuno- suppressant drugs to prevent the donor cells from attacking his body. The transplant was successful and, as hypothesized, the newly reconstituted CD4 T-cells lacked CCR5 receptors. But almost a year later, the man had a relapse of leukemia. The same donor was persuaded to part with more bone marrow, and the patient received a second transplant after chemotherapy and whole-body radiation.

The man stayed off ART, and since two months after the first procedure has maintained undetectable plasma HIV RNA and undetectable proviral DNA in resting CD4 T-cells. Hütter presented this Berlin success story at CROI 2008 and in the February 12, 2009, New England Journal of Medicine. The case sparked interest from both HIV researchers and the public at large after an in-depth article by Schoofs in the Wall Street Journal. In an update at the IAS Reservoirs workshop and in the March 10, 2011, issue of Blood, Hütter's team reported that four years after the first transplant and still off ART, the man remains in remission from leukemia and shows no signs of HIV. Using the best available technology, Siliciano and others have found no HIV RNA or DNA in his blood plasma, lymph nodes, rectal mucosa, cerebrospinal fluid, brain tissue, or resting CD4 T-cell samples. What's more, his CD4 T-cell count has increased to a normal level. A few months after the Vienna meeting, this Berlin Patient revealed his identity as ***Timothy Brown****, now in overall good health and living in San Francisco. While it is not possible to prove that Brown has no remaining HIV anywhere in his body—or whether its disappearance is due to the CCR5-delta-32 stem cell transplant, strong chemotherapy, a graft-vs-host reaction, the anti-inflammatory effect of immunosuppressant drugs, or some other unknown factor—he appears to have achieved a sustained functional cure. (****The New England Journal of Medicine ,February 12, 2009)***

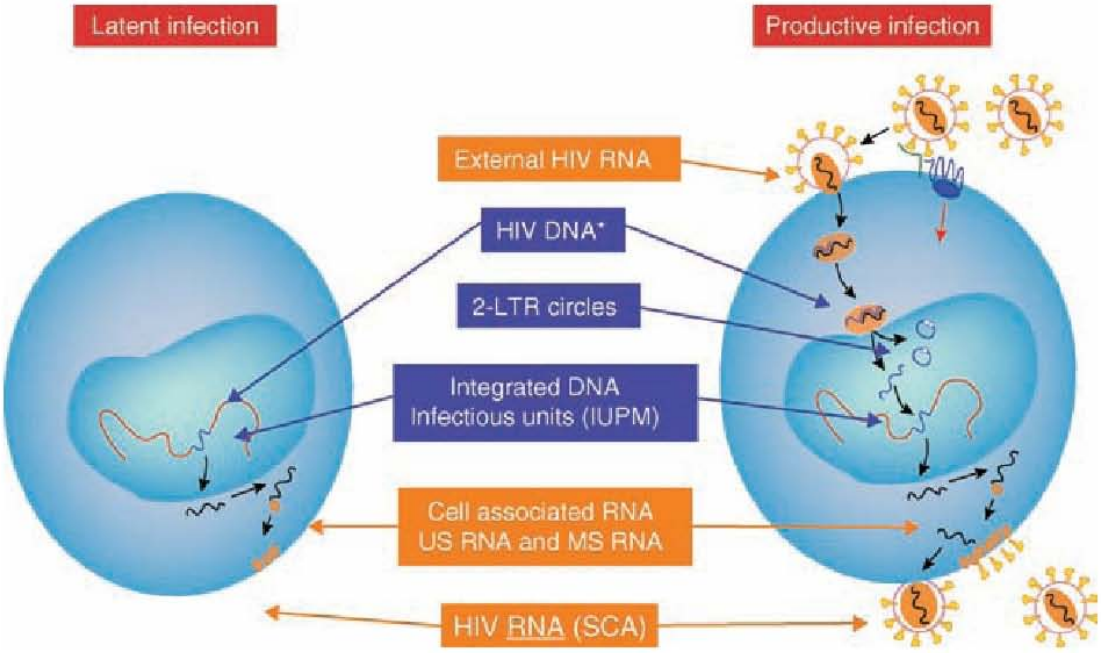

Fig. 1. The viral life cycle in a latently infected cell (left) and productively infected cell (right) is shown together with the currentavailable tests that quantify different forms of HIV RNA (orange boxes and arrows) and HIV DNA (blue boxes and arrows). HIV DNA is shown in blue and HIV RNA is shown in black. _HIV DNA measures unintegrated and integrated DNA as well as 2-LTR circles.