

ABSTRACT

The effectiveness of highly active antiretroviral therapy (HAART) has brought about a paradigm shift in transforming HIV infection into a chronic manageable disease. HAART is required to be given life long which needs good adherence as HIV provirus integrated within the infected cells cannot be eliminated and virus replication resumes following its discontinuation. It is well established that HAART is associated with drug toxicities, drug-drug interactions and multiple co-morbid complications like early ageing due to immune activation and inflammatory phenomena. Long term medication with antiretroviral drugs also leads potentially to diseases of cardiovascular, neurocognitive, kidney, liver, and selection of drug resistance viruses which leads to limitation of prolong therapy. To avoid and overcome the inherent limitations of HAART a series of trials and treatment modalities have been tried with a hope to attempt cure of HIV infection. Genetic association studies have served as a powerful means to identify host factors that influence HIV-AIDS pathogenesis in vivo. The most dramatic example of a genetic factor influencing HIV infection and/or pathogenesis relates to the gene that codes for CC chemokine receptor 5 (CCR5), the major HIV co-receptor for cell entry. Gene manipulations could eliminate latent viral reservoirs in HIV infection and further, prevent infection in newly exposed individuals. Gene therapy strategies are being studied with the endeavour to find cure for HIV.

INTRODUCTION

HIV infection continues to be a major global public health issue with more than 35 million people living with HIV (PLHIV) worldwide.¹ In spite of its effectiveness, HAART for HIV disease does have a number of limitations. Anti-retroviral drugs do not fully restore health. Chronic inflammation and immune dysfunction often persist indefinitely during ARV treatment leading to increased number of AIDS morbidity and mortality. It does not fully suppress the viral replication as cryptic viral replication persists within dispersed haemato lymphoid organs with potentially significant effects on T cells, myeloid cell homeostasis and function (2). It needs strict adherence to regimes. Even with the massive global investment in HIV cure, access to these drugs will remain incomplete and thus epidemic will continue to spread. Destruction of the immune system by HIV is driven by the loss of CD4 T cells in the peripheral blood and lymphoid tissues. HAART controls the HIV replication and allows the immune system to partially restore and delays disease progression but cure

of HIV infection still remains unachievable with use of the currently available ARV drugs. As ARV cannot eradicate provirus present as reservoirs in latent infected cells as HIV virus will reseed the body once ART is discontinued. Viral entry into CD4T cells is mediated by the interaction with a cellular chemokine receptor, the most common of which are CCR5 and CXCR4. Subsequent viral replication requires cellular gene expression followed by depletion of activated memory CD4 T cells most of which reside in GI mucosa (3). Most viruses isolated from individuals shortly after sero-conversion and during asymptomatic phase of infection are using CR5 co-receptor while CXCR4 co-receptor using viruses are seen in late stage of infection and are associated with a rapid disease progression and development of AIDS.

As HAART cannot completely eradicate HIV, multiple strategies for HAART free treatment are approached by many researches with the aim of achieving (i) sterilizing cure of HIV -eradication and elimination of all replicative competent viruses, (ii) functional cure -undetectable plasma viraemia and (iii) building a host cell which is able to resist initial HIV infection. Since 2009 there is growing interest in development of potentiality curative approaches for HIV infection. An ideal therapeutic cure would be one that is safe, scalable, administered for a limited period of time and prevents infection of all susceptible cells including cells in tissues.

CCR5 is the major co-receptor found in T-cell subsets and is utilized by HIV to gain entry into targeted cells. It is well established that Individuals lacking functional CCR5 due to the naturally occurring CCR5 Δ 32 mutation homozygotes are highly resistant to HIV infection and AIDS. Genetic manipulative strategies to make CCR5 receptor in T cells non-functional with an endeavour to prevent attestation of HIV to targeted cells is the basis for the realistic search for cure of HIV.

Inspiration from the Berlin patient

Mr. Timothy Ray Brown - a case of HIV seropositive from Berlin who suffered from acute myeloid leukaemia in whom HIV virus remains undetectable after allogenic stem cell transplantation from a donor having homozygous for the CCR5 Δ 32 mutation following aggressive cytoablative chemotherapy (cyclosporine). HAART was stopped before the transplantation. His serum did not show the presence of HIV up to 7 years after transplantation.^{4,7} This well documented first case of HIV cure gives rise to a hope for new strategies for eradication of HIV infection.

Gene therapy for HIV infection was first proposed by

54 David Baltimore in 1988 with the term “intracellular immunisation” with an intention to make HIV target cells resistant to virus infection by using anti HIV genes. In 2007 Gero Hutter an oncologist and haematologist performed the procedure of transplantation for the Berlin patient providing for the first time the real “proof of the concept” of genetic manipulation for the cure of HIV. HIV resistant phenotype is obtained in individuals with the $\Delta 32$ mutation following hematopoietic stem cell transplantation. This gene modified cells are introduced into the patient safely and efficiently. These findings have opened up treatment to many more HIV-infected patients and Sangamo Bio Sciences. Inc has recently taken this approach and applied it to HIV-infected patients. In the most recently published Phase 2 clinical trials, infusion of zinc finger nuclease CCR5 modified autologous CD4 T cells (SB-728-T) has been shown to increase CD4 counts and decreased HIV pro-viral load in HIV infected patients when ARVs are withdrawn during treatment interruption. This findings and the experience with the Berlin patient validates use of genetically modified early cytokines as an effective strategy in finding a “functional cure” for HIV.

Strategies of gene therapy

Gene therapy is focussed on three major steps:

1. Blocking HIV entry using transduced cells with modified HIV receptors.
 - a. Blocking CD4 binding
 - b. Blocking co-receptor binding and expression
 - c. Blocking membrane fusion
2. Producing disruption or inactivation of Pro-virus using specific endo nucleases
3. Inhibiting the expression of integrated genome with RNA decoys.
 1. Blocking HIV entry into Host cells
 - 1a. Blocking CD4 binding: CD4 plays a crucial function in cellular immunity; Abrogation of CD4 expression is not possible to prevent HIV infection since the final result may lead to lethal immune deficiency in a given patient. Early gene therapy for this technique was done by introducing chimeric T cell receptors (TCR) in cytotoxic CD₈T cells allowing them to recognize and kill HIV infected cells. Following promising pre clinical studies two clinical trials was done to investigate the effects of adoptive transfer chimeric TCR-modified CD4+ and CD8+ T cells on HIV infection. In both trials the genetically modified cells successfully engrafted and trafficked to the rectal mucosa, a major site of HIV replication. Both studies however did not show significance reduction in viral load in treated individuals.⁹
 - 1b. Blocking co-receptor binding and expression: The apparent cure of the Berlin patient after receiving HSCT from a CCR 5 Δ 32 homozygous donor have

inspired attempt to obtain HIV resistant cells through gene therapy. One of the most promising strategies has aimed to disrupt the CCR5 gene by expressing an engineered zinc-finger nucleus (ZFN). ZFNs are an artificial implanted DNA at specific sites in humanised models. It can deliver adenoviral or retroviral vectors or nucleofection. These genetically modified cells are transferred back into the autologous donor. It can inactivate CCR5 in CD4-T cells and CD34+ hematopoietic stem cells limiting HIV replication.¹⁰ Following this study site specific modification of CCR5 gene was made ex vivo, autologous modified CD4+ T cells & subsequently infused back to corresponding patients. These cells harboured a CCR5 gene modified using a ZFN (SB-728-T) to make dysfunctional CCR5 co-receptor. These genetically primed cells can persist in-vivo with a half-life of nearly year and this procedure was safe and the cells could protect from HIV infection.¹¹ To emulate the Berlin patient, the use of cyclophosphamide as a immunomodulatory agent prior to infusion of SB-728-T-1002) is currently being evaluated in ART naive HIV infected individual. There is inherent risk of increase in the percentage of X4 tropic virus which occurs after transplantation most likely driven by pre-existing X4 tropic minority variants. This case highlights the fact that viral escape mechanisms might jeopardize CCR5 knock out strategies to control HIV infection. Thus ZFNs have been designed to simultaneously target CCR5 & CXCR4 as a pilot study showed primary CD4 T cells were resistance to both R5 and X4 viruses.¹³

- 1c. Blocking membrane fusion: The final step for HIV entry into target cell is membrane fusion. HIV gp4 is largely responsible for membrane fusion. It contains two heptad repeat domains (HR1 & HR2) downstream of the N-terminal fusion peptide. Enfuvirtide (T20) was the first entry inhibitor approved for HIV treatment. C46 member anchored form is another gp41 mimetic peptide also inhibit HIV entry. Safety and a modest antiviral effect were recognized in a phase 1 clinical trial. A study on animal model using pigtailed macaque model mC46 for modelling functional cure strategies was presented at CROI 2014. Following these encouraging preclinical data, a phase I/II clinical trial named safety study of a dual anti HIV gene transfer construct to Treat HIV1 infection (clinical trials gov NCT 01734850) looked at the experimental gene transfer Cal-1 (LVsh 5/C46). This agent is designed to inhibit HIV infection by removing CCR5 from bone marrow & peripheral blood mononuclear cells and also by producing C46 that mimics of gp41. Amino acid peptide from the C terminal heptad repeat -2 domain of gp41, named C34 has been designed for inhibition of CCR5 & CXCR(4) HIV+ tropic strain.¹⁴ Engineering primary CD4 + T cells provides trans-dominant

and heterologous resistance to diverse HIV-1 isolates. Cross-clade protection leads to survival and selective expansion of C34COR HIV resistant, functional CD4+T cells that can be expanded ex-vivo and adoptively re-infused represents a promising and innovative approach with the potential to control HIV infection in humans.¹⁵

2. Producing disruption or inactivation of Pro-virus using specific endo nucleases: Endonucleases could disrupt and inactivate gene expression from an integrated lentiviral reporter provirus. Tre-recombinase can excise integrated HIV from host cell chromosome and has shown potent antiviral effect. There is a need for focus on development of rare cutting endonucleases that can target essential HIV genes.¹⁶ Endonucleases like TALENs & CRISPR-Cas could disturb & inactivate gene expression from an integrated lentiviral receptor protein.²⁰
3. Inhibiting the expression of integrated genome with RNA decoys: RNA base factor approaches like antisense RNAs, RNA decoys, ribozymes, aptamers and sh/siRNAs have investigated as anti HIV gene therapy. It finds the target protein due to their three dimensional structure.¹⁷ Two RNA decoys have been tested to inhibit HIV replication. Those are 1. Rev response element (RRE) and 2. Trans-activating region hairpin at the 5-end of viral mRNA transcripts that binds viral Tat protein. HIV provirus has several essential genes and a long single-reading frame gag-pol transcript encoding multiple proteins thereby giving many excellent targets for gene disruption. Further the infected CD4 cells in majority have only one provirus copy suggesting that gene disruption might only need to target one provirus per infected cell. Therefore, a provirus directed anti-HIV agent could be the clue toward HIV cure.

CONCLUSION

Antiviral drugs have been successful in containing HIV infection and making it a chronically manageable disease, yet it cannot achieve complete eradication of the virus from the reservoir. The pursuit for a cure for HIV is making significant stride. Gene therapy proves to be a unique and realistic option as clinically proven by Berlin patient. There are many strategies of manipulating gene by targeting multiple stages of viral cycle with an attempt to disrupt and/or inactivate the genetically reserve viral material in HIV patients to ultimately achieve functional or possibly sterilizing cure. HSCT from donor with 32bp mutated CCR5 receptor or gene editing using nucleases are approaches being studied with encouraging results. Gene therapy may hold the key for a cure for HIV.

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