Libyan International
Medical University
Faculty of Basic Medical Science

CRISPR/Cas9-Based Gene Editing in HIV-1/AIDS Therapy

By: tamiem elzway 1829 3rd year medical science

Supervisor: Dr.khadija mansouri

assisted by: Dr.mohammed alhaji

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Abstract

HIV is one of the main infectious diseases that infect millions of people around the world and the major cause of AIDS, even though great efforts have been made in the prevention and therapy of HIV-1 infection, HIV-1/AIDS remains a major threat to global human health. Highly active antiretroviral therapy (HAART) can suppress virus replication, but it cannot eradicate latent viral, making the HIV-1/AIDS a chronic and persistence of the virus in latent reservoirs, which includes macrophages, microglia, astrocytes, intestinal lymphoid cells, and, mainly, CD4+ memory lymphocytes and failure of patient adhesion to treatment. More Recently scientist found powerful genetic tool called the clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated nuclease 9 (Cas9) system has been engineered as a more effective gene-editing and cheaper technology with the potential to treat HIV-1/AIDS by targeting the long terminal repeats and the Gag gene for elimination of latent virus infection without off-target effect, which are excised in Vivo using infected mice. The integrated Pro-viral DNA elimination confirmed by digital droplet PCR with no off-target effect is detected. These data provide proof of concept that permanent viral elimination is possible without viral rebound.

Introduction

The human immunodeficiency virus (HIV) is the cause of acquired immunodeficiency syndrome (AIDS) belongs to the lentivirus subgroup of retroviruses which formed by single-strand RNA molecules. The complete genome is flanked by two long terminal repeat (LTR) sequences and it encodes 10 viral proteins including gag, pol, vif, vpr, vpu, env, tat, rev, nef, and the antisense protein (ASP), which have different functions in virus invasion and replication [(1)]. There are two different types of HIV, HIV-1, and HIV-2. They both have many similarities and both can lead to AIDS. Compared to HIV-1, HIV-2 has lower transmissibility and is less pathogenic. HIV-1 is recognized as the major cause of AIDS and becomes the main target to prevent and cure AIDS [(2)]. The main virus transmission sources by the unprotected sexual contact, sharing of contaminated syringes and needles, and vertical transmission [3, 4]. It is estimated that in 2017, worldwide, 36.9 million people were living with HIV, with approximately 900000 deaths and 1.8 million new infections [(5)] HIV primary infects the helper T cells (CD4-positive cells) and kills them، resulting in Suppression of cell-mediated immunity which predisposes the host to various opportunistic infections that lead to high mortality rate.

Despite the high incidence and prevalence of the disease, many efforts have been made over the last decades, with particular attention to antiretroviral therapy (ART) to increase survival and reduce hospital admissions, complications due to opportunistic pathogens, and mortality [6, 7]. However, pharmacological therapy is not completely efficiently eliminating latent viral s, making the HIV-1/AIDS a chronic and persistence of the virus in latent reservoirs, which includes macrophages, microglia, astrocytes, intestinal lymphoid cells, and, mainly, CD4+ memory lymphocytes and failure of patient adhesion to treatment [8–9] So, the new therapeutic approaches are necessary.

In last years during development of New genome editing strategies against HIV Initially, tailored recombinases targeting the HIV long terminal repeat (LTR present at both ends of the pro-viral DNA) were developed as a tool to excise the integrated pro-viral DNA from the cellular genome. The pro-viral DNA was also targeted by endonucleases that can be designed to recognize specific DNA sequences, including zinc finger nucleases (ZFN), transcription activator-like effector nucleases (TALEN) and homing endonucleases. These designer nucleases were also used to cleave cellular genes involved in HIV replication, like the gene encoding the CCR5 receptor that facilitates HIV-1 infection (10).

More recently scientist found another important genome more effectively and cheaper genome-editing solution than TALENS and ZFN and less susceptible to off-target effects this solution called CRISPR-Cas genome or (clustered regularly interspaced short palindromic repeat) repetitive sequences of RNA were first observed in 1987 were derive from conjugative plasmids that resist bacteriophages and act as a mechanism of adaptive immunity which responsible for interfere at different stages of the virus infection cycle in the host (11). CRISPR-Cas genome and based on the editor emerges as a powerful tool with believed to create the revolution for treatment for most of genetics disease in feature not only HIV virus.

Aim of study to show how can CRISPR-Cas9 genome inhibit replication of HIV viruses on vivo of mice.

Methods and material

HIV-1 infection of CD34 + humanized mice. the mice were obtained from the Jackson Laboratories from the University of Nebraska Medical Center (UNMC). CD34 + hematopoietic stem cell (HSC) were enriched from human cord blood or fetal liver cells, CD34 + cell purity was >90% by flow cytometry, were the Cells transplanted by intrahepatic injection into newborn mice irradiated of

50,000 cells/mouse in 20. Humanization of the animals was affirmed by flow cytometry for the presence of human CD45 and CD3 positive blood immune cells [12-13]. At 18 weeks of age mice were infected intraperitoneally (i.p.) with HIV-1[14-15] only after weeks of infection we do observe significant cell loss, the viral loads and CD4 + T cell depletion levels observed in our infected humanized mice are in point of fact reflective of the disease course in infected human host [16-17-18-19-20].

The 29 infected humanized mice were divided into four. The first group (n = 6) of mice were left untreated (HIV-1 control), the second group (n = 6) received a single intravenous (IV) injection of AAV9-CRISPR-Cas9, the third group (n = 10) were administered LASER ART by intramuscular (IM) injection. A fourth group (n = 7) received LASER ART followed by AAV9-CRISPR-Cas9 (combination treatment).

Result

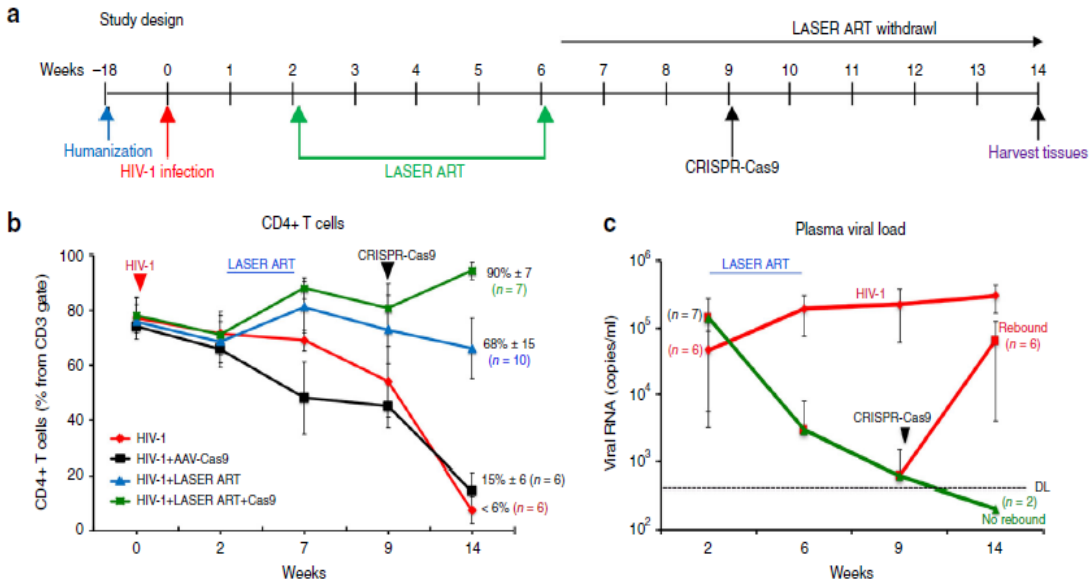

Fig. 1 Viral load and CD4 + T cells in HIV-1 infected and treated humanized mice. Mice were infected with 104 TCID50 of HIV-1NL4–3 followed by treatments with LASER ART, CRISPR-Cas9 or both a) The study scheme shows the times of infection and treatments. Flow cytometry for human CD4 + T cells are shown (b) with increased numbers of CD4 counts in the LASER ART and dual LASER ART and CRISPR-Cas9 groups. (c) Evaluation of plasma viral load indicated that after administration of AAV9- CRISPR-Cas9, 2 of 7 mice showed no evidence for viral rebound at 14 weeks.

Accordingly to the results in the current study verified the bioavailability of our gene editing molecule in various organs of the NSG humanized mice which improves CD 4 count and prevention of the viral rebound

Discussion

While ART has transformed HIV-1 infection into a chronic treatable disease but the virus still persists in tissues that include the gut, lymph nodes, brain, spleen, and amongst other sites. The inability of ART to eliminate virus in these tissue remains the major obstacle towards a disease cure. For elimination of proviral DNA, we chose the CRISPR-Cas9 gene editing platform and created a multiplex of gRNAs that caused cleavage of the viral genome at the highly conserved regions within the LTRs and the Gag gene [21-22-23].

This genome editing tool derived from bacteria and consist of a guide RNA (gRNA) which is complementary target DNA sequence, and endonuclease (Cas9) which make single or double strand break at target site [24]. This Break imperfectly repaired by nonhomologous end joining (NHEJ) which carry risk of frameshift mutation and that why it not used clinical because it lead to genetic variation but in this study, the CRISPR gene was very specific which no detectable off target effects [24].

In order to make sure the delivery CRISPR-Cas9 gene into multiple tissue including central nervous system, the sequence verified plasmid was sent for packaging into adeno-association virus serotype 9 was chosen as the vector for CRISPR-Cas9 delivery [25-26]. Results from ddPCR showed 60% to 80% efficiency of viral DNA excision by CRISPR-Cas9. Of note, this approach quantified dual cleavage events that removed the DNA fragment spanning.

Conclusion

They have employed a highly sensitive tests to evaluate HIV-1 elimination by LASER ART and AAV9 delivered CRISPR-Cas9 treatments on mice. These included viral gene amplification, flow cytometry, adoptive viral transfers, and measures of viral rebound with CD4 count to demonstrate at combination therapies can safely lead to the elimination of HIV-1 infection. Results demonstrated that eradication of replication-competent HIV-1 present in infectious cell and tissue sites of infected animals can be achieved.

The Future studies are designed to improve delivery of agents to viral reservoirs and specifically eliminate residual latent viral infections. This is a first important step towards a longer journey for viral eradication.

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