CRISPR/Cas9 mediated intervention to target the host CD4$^+$ T cells that are latently infected with HIV-1 genome.

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HIV/AIDS has been a global health issue since the 80's claiming human lives each year worldwide. Normally the anti-retroviral therapy (ART) is robust to clear the viral load. However, the viral latency still causes relapses and challenges ART. In order to identify the latent viral reservoirs, viral genome containing host cells and re-activate the viral replication or permanently silence the viral genome, we hypothesize that a CRISPR/Cas9-based intervention can be devised. Multiple guide RNA molecules were designed to target the viral genome. These guide RNA molecules are yet to be evaluated in future.

Human immunodeficiency virus type-1 (HIV-1) infection targets the CD4$^+$ T cells of the host immune system. Rapid and error-prone replication of HIV-1 gradually reduces the number of such CD4$^+$ T cells thus weakening the host immune system. In such individuals, due to compromised immune system, multiple opportunistic infections including Kaposi's sarcoma may further lead to the acquired immunodeficiency syndrome (AIDS). It is much better to start the anti-retroviral therapy (ART) to treat the HIV-1 infection before the patients' health condition worsens to AIDS. ART contains a combination of the viral reverse transcriptase inhibitors, integrase inhibitors, protease inhibitors and fusion inhibitors. ART can only target the virus when there is active viral replication. HIV-1 is known to incorporate its genome into the host genome and exhibit latency for long periods of time (1, 2). In such cases, even if the patients recover on ART, relapses often cause challenges due to the activation of latent viral replication. As of today there are no medications to treat the latent HIV-1 infections to prevent such relapses. Hence new approaches are needed immediately that can be combined with ART.

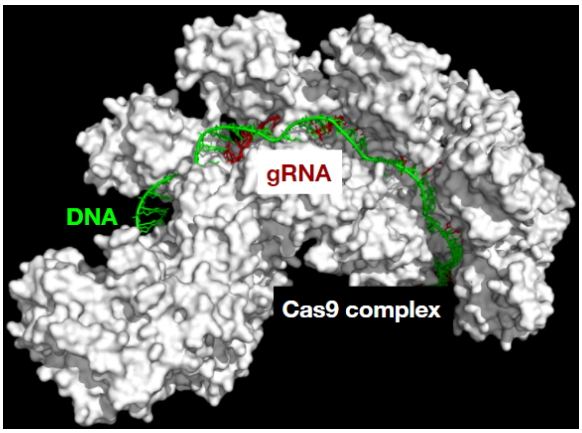

Figure 1. Structure of Cas9 (white color) in complex with DNA (green color) complementary to the guide RNA (red color) downloaded from the protein data bank (PDB ID: 4QYZ).

CRISPR (clustered regularly inter-spaced short palindromic repeats)/Cas9 methodology has been a very nifty genome editing tool with precision (3). In this communication, we hypothesize that by using specific guide RNA (gRNA) molecules, one can target the latently infected host cells in effectively treating HIV-1 infections to potentially avoid any future relapses. Accordingly six different locations in the HIV-1 genome were chosen for the gRNA design viz., gag, pol, env, nef and both of the long terminal repeats (LTRs) on the 5' and 3' ends.

Each of the six gRNA molecules were designed in such a way that the total length of the spacer region in each molecule is around 20 bases starting from the protospacer adjacent motif (PAM). The length of 20 bases was chosen to achieve specificity as well as to avoid off target binding. By using a combination of these gRNA molecules, the latently infected human T cells can not only be identified but also can be successfully targeted for genome editing to remove the incorporated viral genome. Currently the gRNA molecules are under evaluation *in vitro* and *in vivo* to understand their specificity. These gRNA molecules along with the Cas9 will be further delivered in liposomes to target the $CD4^+$ T cells to identify and edit the genomes of latently infected cells.

In future, the same technology will be used to evaluate the gene silencing in the latently infected T cells through genetic and epigenetic targeting. The data obtained from the *in vitro* and *in vivo* studies will be published in the future issues of TCABSE-J.

References

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