RNA-Guided Genome Engineering for HIV Resistance

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Two major research initiatives for potentially eradicating HIV/AIDS are (1) to eliminate the reservoirs of latently infected cells and (2) to create an HIV-resistant immune system. In this research proposal, we will use a recently discovered biological system, known as CRISPR, to explore each of these two strategies. This project will include studies to evaluate the anti-HIV efficacy, targeted delivery, on-target specificity, and safety of the CRISPR system.

CRISPR is a natural gene-editing process that functions as an immune system in certain bacteria by causing mutations in the DNA of invasive viruses. This system uses a DNA-cutting enzyme that is guided by a small RNA to recognize and cut a specific target DNA sequence, analogous to a 'lock-and-key.' We aim to harness the CRISPR gene editing technology as a new anti-HIV therapeutic that may offer a potential curative strategy for HIV. In this project, we will engineer the CRISPR system to target harmful HIV genes as well as human *CCR5* and *CXCR4* genes, which encode cellular co-receptors required for HIV infection.

A major obstacle in the search for an HIV/AIDS cure is the elimination of latently infected T-cells, in which the virus can remain dormant for years or even decades despite continuous antiretroviral therapy. It is well established that the vast majority, if not all, HIV-infected adults have a reservoir of latent virus that will likely persist the rest of their lives. We will utilize the CRSIPR system as a new anti-HIV therapeutic by targeting the HIV genome in latently infected cells, which is not achievable with current therapeutics. This technology is readily adaptable to target different HIV genes or even different DNA sequences within the same HIV gene by simply changing the sequence of the small RNA.

Using the same CRISPR system with different small RNAs, we will direct DNA damage in specific human genes that are necessary for HIV infection, but are not essential for normal cell health. One such gene is called *CCR5*. People with natural mutations in this gene have normal health, but they are also naturally immune to many different HIV strains. Thus, we propose using the CRISPR technology to disrupt the *CCR5* gene in hematopoietic stem cells, which could generate an immune system that is resistant to HIV infection. This concept is supported by the recent report of the famous 'Berlin Patient,' who was functionally cured of HIV after receiving a transplant of hematopoietic stem cells that contained natural mutations in the *CCR5* gene. However, natural mutations within the *CCR5* gene are extremely rare, so a technology that could specifically eliminate the *CCR5* gene might vastly expand the number of HIV patients that could be functionally cured like the 'Berlin Patient.' In addition to *CCR5*, we propose a similar CRISPR strategy to remove the human *CXCR4* gene in T-cells. Like CCR5, CXCR4 is human co-receptor used during HIV infection.

Finally, we will investigate the safety of the CRISPR system in a small animal model and explore methods of delivering the CRISPR system to human cells, including the usage of viral vectors, RNA aptamers, and dendrimer nanoparticles. Together, these experiments are designed as a pilot study to develop the CRISPR gene-editing technology as a potential therapeutic to eliminate latent HIV infections and to create HIV-resistant immune cells.