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TAG
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Gene Editing in HIV Research

INTRODUCTION

The term "gene therapy" refers to a broad variety of approaches that aim to provide therapeutic benefit by manipulating genetic code (DNA or RNA). The strategy takes advantage of the fact that DNA acts as a blueprint for manufacturing proteins, via an intermediate step of translation into RNA. Multiple research groups and biotechnology companies have explored the possibility of treating HIV with gene therapy, but so far evidence of efficacy has been limited, and no candidates have been submitted to the U.S. Food and Drug Administration (FDA) for possible approval. Importantly, no major safety issues have emerged to date.

The purpose of this issue brief is to provide background information on gene editing in HIV research, and context for the news about He's misuse of the technology to alter embryos.

Initially, these experimental gene therapies involved introducing pieces of genetic code designed to make proteins capable of exerting anti-HIV effects. For example, in one trial a gene therapy was used to equip infection-fighting T cells with a receptor intended to enable them to better recognize HIV.

More recently, technologies that allow editing of genetic code have entered the picture. Rather than introducing a gene intended to manufacture a particular protein, gene editing has the capacity to alter existing genes in beneficial ways.

Gene editing gained particular notoriety toward the end of 2018 due to extremely controversial claims that it had been used in China to alter embryos, leading to the birth of twin babies with disrupted CCR5 genes. CCR5 is a protein receptor that most strains of HIV latch onto in order to infect cells, and the researcher involved, He Jiankui, said he was trying to create resistance to HIV infection. He has since been fired from his position at the Southern University of Science and Technology in Shenzhen and placed under investigation by Chinese authorities.

Amidst the controversy about He Jiankui, it's important to appreciate that there is a substantial amount of legitimate and responsible gene editing research in HIV. The best-known example is an experimental therapy called SB-728, developed by the company Sangamo Therapeutics. SB-728 is designed to edit the gene that encodes the CCR5 receptor, with the aim of creating cells impervious to infection by HIV. Several other gene editing approaches that may have benefits in HIV are also under investigation.

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GENE EDITING APPROACHES IN HIV RESEARCH

Various technologies have been developed that have the capacity to edit genetic code (see box). The general mechanism involves targeting a location in the genome and delivering proteins that cause a break in the DNA at that location. Natural repair mechanisms then mobilize to mend the break, but the gene at the targeted location can be disrupted.

Gene editors can be used to cause DNA breaks at two locations, leading to the removal of all genes between the targeted sites. They can also be paired with methods that cause the repair mechanisms to insert alternate genetic code, such as a therapeutic gene or a functional version of a dysfunctional gene.

Leading Gene Editing Technologies

- Zinc finger nucleases (ZFNs)
- Transcription activator-like effector nucleases
- Meganucleases
- Clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9 (CRISPR/Cas9)

SB-728

In the example of SB-728, zinc finger nucleases (ZFNs) are employed to disrupt the CCR5 gene in cells extracted from people with HIV. The cells are then infused back into the same person with the goal of generating a cell population resistant to HIV infection.

The rationale for targeting the CCR5 gene derives from knowledge of a rare natural human genetic variation called the CCR5Δ32 mutation. People who inherit CCR5Δ32 from both parents—homozygotes—lack a functional CCR5 receptor and are protected against most strains of HIV (although they can be vulnerable to viruses that enter cells via an alternate receptor, CXCR4).

Timothy Ray Brown, who is considered cured of HIV infection, received stem cells from a donor who is homozygous for the CCR5Δ32 mutation as part of treatment for cancer over a decade ago. These cells generated a new immune system lacking functional CCR5 receptors, and this is believed to have played a key role in his remarkable case. Ongoing studies are providing stem cells from donors homozygous for the CCR5Δ32 mutation to people with HIV and cancers, in hopes of achieving additional cures (two new cases similar to Brown were reported in March 2019).

SB-728 has been extensively studied in people with HIV.

SB-728 has been extensively studied in people with HIV. In most clinical trials, CD4 T cells—the primary target cell for the virus—have been extracted from the blood of HIV-positive participants, exposed to ZFNs in the laboratory, then multiplied and reinfused into the donor. In one ongoing trial (see table), hematopoietic stem and progenitor cells—which give rise to multiple cell types, including CD4 T cells—are being modified.

Results to date have suggested that the approach is generally safe (there has been one instance of an infusion reaction requiring a brief hospitalization). A few study participants have controlled HIV viral load to relatively low levels after interruption of antiretroviral therapy (ART). CD4 T cell counts increased significantly in most recipients after a single infusion—before declining slowly back toward their baseline level.

The major challenge for the approach is maximizing the proportion of the body's CD4 T cells that are modified to lack a functional CCR5 receptor. A single dose of a chemotherapy drug, cyclophosphamide, has been used in many trials to try to deplete existing CD4 T cells before infusion and make more room for the modified cells. However, this has not increased their levels enough to lead to control of HIV in the majority of participants.

At this point, Sangamo Therapeutics does not appear to believe that the efficacy is sufficient to justify further commercial development, but the product is being supplied for additional investigator-initiated studies (see table). HIV advocates have argued that SB-728 might have a role in promoting CD4 T cell reconstitution in people who experience suboptimal immune recovery despite ART because of encouraging results in an early trial, but the company has not shown interest in pursuing this indication.

CRISPR/Cas9

A different, more recently discovered gene editing technology is CRISPR/Cas9 (short for clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9). CRISPR/Cas9 is derived from a type of primitive immunity present in bacteria that evolved to fend off invading bacteria or viruses. CRISPR/Cas9 has become popular with scientists because it is cheaper, easier to use, and more precise than previous methods.

In laboratory studies, CRISPR/Cas9 has been successfully targeted to two locations in HIV DNA, at either end of the virus's genome, causing the excision of all the virus's genes from infected cells. Integration of HIV DNA into the DNA of infected cells is considered the major obstacle to curing HIV, so these laboratory results have generated considerable excitement and media coverage.

The idea of targeting HIV DNA for removal is not new, and it has been attempted with older gene editing techniques such as meganucleases, but CRISPR/Cas9 appears to be better suited to the task than previous methods.

Work is underway to assess whether the approach can be translated into a therapy for testing in humans, but there are significant challenges that will need to be addressed.

A critical first step is developing a strategy for safely delivering the CRISPR/Cas9 gene editing machinery to cells containing integrated HIV DNA, which are distributed throughout the body. Because CRISPR/Cas9 is derived from bacteria, there is a risk that it might provoke an immune response that would interfere with delivery—this problem has been described in mouse studies.

Another challenge is that studies have found that HIV can develop resistance to CRISPR/Cas9, which will likely make it necessary to target multiple sites in the viral genome (somewhat similar to the rationale for combination therapy with ART).

A major concern with all gene editing approaches is the potential for off-target effects. Inadvertent damage to other genes could present serious problems, potentially triggering cancer development, for example. Careful surveillance for any off-target effects will be central to establishing safety.

A rare phenomenon in which more than one copy of the HIV genome integrates into the DNA of an infected cell could also pose difficulties for efforts to excise the virus. If CRISPR/Cas9 were to make a cut at each end of the genome of two separate integrated viruses (rather than a single virus), it's possible that all the human genes between the two HIV DNA copies would also be excised, severely damaging the cell genome.

To date, clinical trial registries indicate that just one study involving the therapeutic use of CRISPR/Cas9 has been initiated for HIV (see table). Researchers in China are investigating whether the approach can be used in the laboratory to disable the CCR5 gene in hematopoietic stem cells, which will then be administered to people with HIV who require stem cell transplants to treat cancers. The goal is to ascertain whether the effects of the CCR5Δ32 mutation can be mimicked, potentially leading to the salutary outcome seen in Timothy Ray Brown.

CURRENT HIV CLINICAL TRIALS INVOLVING GENE EDITING APPROACHES

Trial	Trial Registry Identifier	Sponsor	Phase	Estimated Completion Date
Gene Therapies				
SB-728-T modified T cells	NCT03666871 (not yet open for enrollment)	Case Western Reserve University	Phase I/II	February 2024
CD4 CAR + C34-CXCR4 + SB-728mR modified T cells	NCT03617198 (not yet open for enrollment)	University of Pennsylvania	Phase I	December 2025
SB-728mR modified hematopoietic stem and progenitor cells	NCT02500849 (closed to enrollment)	City of Hope Medical Center	Phase I	March 2019
SB-728mR modified T cells	NCT02388594 (closed to enrollment)	University of Pennsylvania	Phase I	February 2019
Gene Therapies for HIV+ People with Cancers				
CRISPR CCR5 modified CD34+ stem cells	NCT03164135	307 Hospital of PLA (Affiliated Hospital to Academy of Military Medical Sciences in China)	Not listed	May 2021

HE JIANKUI'S CLAIMS OF GENE-EDITED BABIES

CRISPR/Cas9 is also the technique that researcher He Jiankui claims to have used to disrupt the CCR5 gene in embryos for in vitro fertilization, leading to a pregnancy and the birth of twins. He's announcement was made via several YouTube videos in November 2018, leading to massive media coverage and an international furor.

He subsequently gave a presentation at a conference, providing some background on the claim. Heterosexual couples with an HIV-positive male partner and an HIV-negative female partner who wanted to have children were recruited for what He misleadingly described as a study related to HIV vaccination. The couples were offered in vitro fertilization (IVF), which is not accessible for most people with HIV in China. Before implantation, embryos were edited with CRISPR/Cas9 targeting the CCR5 gene.

Independent scientists who have looked at the data He presented say that the exact nature of the gene edits—and their impact on CCR5 receptor expression—is unclear.

The result, according to He, has been the birth of twins with edited CCR5 genes, along with one other successful pregnancy that has yet to reach term. Independent scientists who have looked at the data He presented say that the exact nature of the gene edits—and their impact on CCR5 receptor expression—is unclear. No evidence of off-target effects was reported, but other specialists in the field have questioned the extent of the evaluation.

There is obviously a vast difference between using gene editing to modify cells for therapeutic purposes and attempting to create gene-edited human beings (referred to as germline editing). He's argument is that the experiment was justified in order to allow the families to have children who are resistant to HIV infection, but this rationale is deeply flawed. Among many other issues, the abrogation of CCR5 receptor expression—even if achieved—is not completely protective against HIV, and there could be risks both known (such as increased susceptibility to certain other viral infections) and unknown.

He's study was also profoundly unethical, because it offered the inducement of IVF to participants when it was likely unavailable to them otherwise, and provided an informed consent form that was appallingly inadequate: The purpose of the study and the potential risks were poorly explained, or not explained at all, and a great deal of focus was placed on He's securing rights to photos of any offspring. News reports have suggested that published documents purporting to show appropriate ethical approval were falsified.

Chinese authorities have since declared the experiment illegal. He's trial has been stopped, and investigations are ongoing.

The scientific community both within China and globally has decried the work, and it has prompted a renewed effort to generate updated guidance on the appropriate conduct of research into gene editing.

On February 14, 2019, the World Health Organization announced the launch of the WHO Expert Advisory Committee on Developing Global Standards for Governance and Oversight of Human Genome Editing. The committee is tasked with reviewing the scientific, ethical, social and legal challenges associated with gene editing and making recommendations regarding optimal governance mechanisms. The co-chairs are Justice Edwin Cameron and Dr. Margaret Hamburg, and the first meeting will be held in Geneva in March 2019.

SUMMARY

- Investigation into the potential of gene therapies is a long-standing component of HIV research.
- Gene editing techniques are opening new frontiers for the development of gene therapies and there are strong rationales for pursuing this work in HIV, but research is in the early stages.
- There is currently no justification for attempting to edit the human germline to alter the response to HIV, and the unethical and misguided experiments conducted by He Jiankui should not detract from, or slow, legitimate efforts to develop gene-editing-based therapeutic approaches for HIV infection.

ADDITIONAL READING AND TAG RESOURCES

Treatment Action Group Statement on the Reported Birth of Twins with Edited CCR5 Genes, November 26, 2018
http://www.treatmentactiongroup.org/content/treatment-action-group-statement-reported-birth-twins-edited-ccr5-genes

Research Toward an HIV Cure Trials
http://www.treatmentactiongroup.org/cure/trials

Genome-edited baby claim provokes international outcry
David Cyranoski and Heidi Ledford, *Nature*, November 26, 2018
https://www.nature.com/articles/d41586-018-07545-0

The CRISPR-baby scandal: what's next for human gene-editing
David Cyranoski, *Nature*, February 26, 2019
https://www.nature.com/articles/d41586-019-00673-1

Transplant success reignites interest in reprogramming cells against HIV
Colin Barras, *Nature Medicine*, March 14, 2019
https://www.nature.com/articles/d41591-019-00011-y

The Man Who Smelled Like Rancid Creamed Corn to Usher In a New Scientific Era
Eben Kirksey, *The Atlantic*, December 19, 2018
https://www.theatlantic.com/health/archive/2018/12/hiv-aids-genetic-engineering/578560/

Genome-editing Technologies for Gene and Cell Therapy
Morgan L. Maeder and Charles A. Gersbach, *Molecular Therapy*, February 16, 2016.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4786923/

CRISPR/Cas9 Genome Editing to Disable the Latent HIV-1 Provirus
Amanda R. Panfil, James A. London, Patrick L. Green, and Kristine E. Yoder, *Frontiers in Microbiology*, December 14, 2018
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6302043/

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