

EDITORIAL



Engineering Cellular Resistance to HIV

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A remarkable story of bedside-to-bench-to-bedside research is the discovery of the human immunodeficiency virus (HIV) coreceptor CCR5 and the subsequent therapeutic advances made because of this knowledge. Persons who were heavily exposed to HIV and did not become infected were studied, and a homozygous 32-bp deletion ($\Delta 32/\Delta 32$) was found in *CCR5*, the gene encoding this transmembrane protein.¹ The deletion did not impair CD4 T-cell function, but it did render cells resistant to infection with the typically transmitted R5 viruses that use the CCR5 receptor together with CD4 to gain entry into cells. This knowledge, in turn, led to the development and use of a new class of so-called entry inhibitors that block the CCR5 interaction with the HIV envelope, as well as to an apparent cure of HIV infection, achieved through transplantation of allogeneic stem cells from a donor homozygous for the *CCR5*- $\Delta 32$ deletion.²

Now this knowledge, originally derived from a clinical observation, has led to another important step forward. In this issue of the *Journal*, Tebas et al.³ report on the successful adoptive transfer, in patients with HIV infection, of autologous CD4 T cells in which the gene encoding CCR5 is knocked out. In a study designed to examine the safety and tolerability of targeted genome editing in humans with HIV infection, the investigators found that the genetically modified cells persist in vivo with a half-life of nearly a year; they also appear to be protected from HIV infection, because when antiretroviral therapy (ART) is stopped, they are depleted at a slower rate than are unmodified cells.

To knock out *CCR5* in autologous CD4 T cells, the authors used an adenoviral vector constructed

to express a zinc-finger nuclease (ZFN). ZFNs are essentially artificial restriction enzymes that can be designed to cleave DNA at specific sites. Since mutations are commonly induced during the natural repair of these breaks, it becomes possible to disrupt the ability of the targeted allele to make a functional protein, in this case CCR5.

This approach yielded the functional loss of CCR5 on CD4 T cells ex vivo, which were then reinfused. Twelve persons who had HIV infection with undetectable viral loads while they were receiving highly active ART were recruited. In an initial cohort of six persons, ART had restored CD4 T-cell numbers to more than 450 cells per cubic millimeter at the time of screening (these persons were referred to as immune responders); in a second cohort of six patients, CD4 T-cell counts remained between 200 and 500 per cubic millimeter despite treatment with ART for 2 years or more (referred to as immune nonresponders). For each participant, 10 billion autologous CD4 T cells were given as a single infusion after they had been genetically modified with a ZFN to knock out the *CCR5* gene. In the six immune responders, a 3-month treatment interruption was implemented, with careful monitoring, to examine the potential antiviral effects of this approach.

The results from this small study are notable. *CCR5* knockout was documented in 11 to 28% of the cells before reinfusion, and initial CD4 T-cell counts in vivo nearly tripled, with median levels of the genetically modified cells of 250 cells per cubic millimeter 1 week after infusion, constituting approximately one in seven circulating CD4 cells in vivo. Although viremia recurred in all persons in whom treatment was interrupted,

the decline in *CCR5*-modified cells was significantly less than the decline in unmodified cells, and one of four participants who were evaluated had an undetectable viral load at the time of treatment reinitiation. Moreover, HIV DNA decreased in the cells of most of the patients.

The firm conclusion of this study is that genome editing of human cells was safe and associated with an acceptable adverse-event profile and that the cells persisted *in vivo*. The tantalizing question raised by the transient treatment interruption is whether it might actually have been partially effective. A definitive answer to this question will require additional studies.

The current promise and limitations of this and all similar genome-editing technology are based on the efficiency of gene disruption and the propensity for off-target effects. The efficiency is dictated by the particular platform (e.g., ZFN), the targeted sequence, the chromatin DNA structure at the target site, and the vector used to express the genome-modifying nuclease. For this approach to become clinically useful as treatment for HIV infection, biallelic *CCR5* knockout will be required. It was interesting to note that the study participant in whom the viral load fell to undetectable levels was later found to be heterozygous for the *CCR5*-delta32 deletion — and therefore did not require biallelic knockout. Consistent nuclease-mediated biallelic knockdown is not yet tenable.

Off-target effects of the endonuclease are another concern. Off-targeting results from some flexibility in DNA recognition by the ZFN. Non-targeted DNA cleavage is known to occur, but the exact frequency and, more important, the number of events that might result in a mutagenic event elsewhere in the genome, potentially providing a premalignant phenotype, are not truly known. Therefore, increasing efficiency alone may not be sufficient.

Treatment advances in HIV infection over the past few decades have been remarkable, and the long-term safety, durability, and effectiveness (both in terms of cost and outcomes) of genome editing are yet to be determined. This proof-of-principle study is an important first step, not just in the treatment of those infected with HIV but also for genome editing in a broader sense. And further progress can be expected. Although engineered homing endonucleases (meganucle-

ases) and ZFN-based genome editing have an established history, in the past few years there has been an explosion in new technology.⁴ Two newer approaches are transcription activator–like effector nucleases (TALENs) and RNA-guided nucleases based on sequences associated with clustered regularly interspaced short palindromic repeats (CRISPR). A potential advantage of these two systems is that they afford greater design flexibility, making it easier and quicker to identify a set of molecules that will target the desired DNA sequence. But with respect to modifying the target locus, each of these tools on average has a similar efficiency, and currently it is not possible to predict which will be more efficient at a given locus. When these tools are used in the clinic, off-targeting effects are of particular concern. Meganucleases, TALENs, and ZFNs are associated with less off-targeting than are CRISPR nucleases, but this might change as newer refinements in the CRISPR system are further developed.

The potential future of gene knockout by ZFNs and other techniques is not restricted to HIV infection. There are now methods that can be used not only to inactivate a gene but also to make specific nucleotide changes in a specific site in the genome and gene addition. These methods will be useful in fixing genes that contain harmful mutations and in supplying therapeutic proteins. Through repeated trips from bedside to bench and back again, it is likely that these approaches represent a basis for effective future therapeutic interventions.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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