Treatment by CRISPR-Cas9 Gene Editing — A Proof of Principle

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As a tool of great promise for the treatment of inherited human diseases, the clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 nuclease system has captured public imagination, with its precision and versatility being likened to genetic scissors and the letter-by-letter editing capabilities of word-processing software.[1] No longer hypothetical, CRISPR-Cas9 is producing exciting results for medical teams. As described in this issue of the *Journal*, Frangoul and colleagues[2] have used the gene-editing technology to achieve a remarkable level of functional correction of the disease phenotype in two transfusion-dependent patients with either β-thalassemia or sickle cell disease by alleviating the severe anemia and associated medical problems caused by these disorders, which can be agonizing and deadly. Where did this remarkable gene-editing tool come from, how does it work, and how was it applied to achieve the results reported by Frangoul et al.?

Innovation began in observation, when Emmanuelle Charpentier was studying the bacterium *Streptococcus pyogenes* and further characterized the DNA-cleaving component of its primordial immune system.[3] Inspired by that bacterial ability, Charpentier collaborated with Jennifer Doudna in a Nobel Prize–winning project[4] to recreate, simplify, and reprogram this naturally occurring technique to cut DNA molecules at will.[5] This discovery and the subsequent harnessing of CRISPR have enabled greater control than was available through mutations induced by chemicals or radiation,[6] and its relative reliability and affordability compare positively with both traditional genetic targeting and other newer genetic editing innovations.

The CRISPR system evolved in bacteria as an adaptive antivirus immune system.[7] The bacterium uses a primary exposure to virus to capture pieces of the viral genome sequence and incorporate them into CRISPR-related elements of its own genomic DNA, thereby "memorizing" that virus sequence. After subsequent infection with the same or a similar virus, the bacteria use that stored virus sequence to generate an RNA molecule containing the memorized virus sequence and a CRISPR-region sequence that together strongly bind to the Cas9 nuclease protein. The RNA component guides and then tethers the ribonucleoprotein complex to the matching sequence in the bacterial viral genome, whereupon the nuclease component cuts and degrades the virus DNA genome. Subject to certain rules, such as the length of the guide portion of the RNA, this system has been harnessed to precisely bind to a target sequence and cut the genomes of cells of many organisms, including humans.[8-10]

To determine a CRISPR-Cas9–editing treatment strategy, Frangoul et al. synthesized a series of guide RNAs with the goal of finding a sequence that achieves cutting of the target genomic DNA site with high efficiency. The risk is that a sequence that is sufficiently similar to the desired target exists elsewhere in the genome, which results in off-target cutting. However, the authors found high rates of on-target and no off-target editing in a preclinical assay using CD34+ cells from healthy humans. When the CRISPR-Cas9 protein–guide RNA complex creates a double-stranded cut in genomic DNA, the cell responds with intrinsic mechanisms that have evolved to repair DNA breaks. Mostly, this repair results in simple annealing of the break to reproduce the original sequence, but as the CRISPR system keeps cutting, the repairs include imprecise insertions or deletions (indels) of base pairs at the cut site until the CRISPR system no longer recognizes the sequence. The goal is for indels at the target site to eventually disrupt the function of that DNA sequence entirely.

In patients with β-thalassemia or sickle cell disease, the severe anemia and associated medical problems are caused by a failure in synthesizing sufficient amounts of the beta chains of hemoglobin (in β-thalassemia) or by the production of a mutant form of beta chains of hemoglobin called sickle-globin that induce the sickling of red cells (in sickle cell disease).[11,12] Rather than trying to repair the abnormal beta globin gene, Frangoul et al. used CRISPR to dis-

rupt a portion of the erythroid-control element that governs the expression of *BCL11A*, a gene that encodes a DNA-binding protein that regulates switching from the production of fetal hemoglobin before birth to the production of adult hemoglobin after birth.[13,14] In red-cell precursors, *BCL11A* turns on after birth, acting to suppress the production of the gamma globin component of fetal hemoglobin. Inherited mutations in erythroid-control elements of *BCL11A* that are specific to the regulation of expression in red-cell precursors prevent the production of BCL11A protein, thereby permitting the continued production of fetal hemoglobin into adulthood. Patients with β-thalassemia or sickle cell disease who also inherit mutations in erythroid-control elements of *BCL11A* maintain sufficient fetal hemoglobin production to result in a much milder disease phenotype.[15]

Frangoul et al. used CRISPR to precisely cut DNA in that same erythroid-control region of *BCL11A*, thereby inducing high-level production of fetal globin when those modified stem cells engrafted in the patients and differentiated to produce red cells. They performed this technique using patient-derived CD34+ hematopoietic stem cells and then transplanted the cells back into chemotherapy-conditioned patients, which resulted in long-term engraftment with the potential of lifelong production of red cells with high levels of fetal hemoglobin. A critical element of the editing process involved a specialized instrument (an electroporator) that delivers a precise electrical pulse to the hematopoietic stem cells in protective medium containing the CRISPR-Cas9–guide RNA complex, a process called electroporation, which transfers the CRISPR complex into the cells. After electroporation, the cells are allowed to recover, during which time editing occurs. The cells are then cryopreserved to facilitate quality-control analysis before being thawed for infusion.

With tangible results for their patients, Frangoul et al. have provided a proof of principle of the emerging clinical potential for gene-editing treatments to ameliorate the burden of human disease — a documented success in the application of Charpentier and Doudna's groundbreaking tool. Their use of this method may soon be one of many such exciting applications based on a virtual explosion of recent innovations in CRISPR technology.[16]

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