Molecular Therapy
Commentary

Getting Back to Normal: Correcting SCN by Universal or Precision Strikes

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This has been a year full of surprises, but the awarding of the Nobel Prize in Chemistry to Jennifer Doudna and Emmanuelle Charpentier for their discovery of CRISPR/Cas9 editing was not one of them.[1] These genomic scissors are being widely applied in experimental, diagnostic, and therapeutic areas. Of more than 10,000 monogenic diseases, a subset of these affecting blood development and function proffers the most fertile ground for proof-of-concept in therapeutics. In this issue of *Molecular Therapy*, Tran et al.[2] compellingly advance CRISPR-Cas9-mediated repair of a missense mutation in *ELANE*, the most commonly affected gene causing severe congenital neutropenia (SCN).

SCN is a life-long deficiency of neutrophils, which serve as the primary defense against infections.[3] Children typically present within the first several months of life with life-threatening bacterial infections of the skin, gut, or lungs. There is a near absence of neutrophils (normal range is between 1,500 and 7,500 neutrophils/μL) owing to a maturation arrest at the promyelocyte stage of neutrophil production. Studying families with cyclic neutropenia 20 years ago, Horwitz et al.[4] identified that the disease was associated with mutations in *ELANE* (*ELA2*), which encodes a neutrophil serine protease. These mutations, which number more than 100, behave in an autosomal dominant fashion. Twenty years later, we still do not understand how this protease causes such havoc. Apoptosis is among the proposed hypotheses due to unfolded protein response and/or mislocalization or impaired differentiation.[5]

The pharmacologic use of granulocyte colony-stimulating factor (G-CSF) increases neutrophil counts to protective levels in the majority of SCN patients. However, the growth factor may promote transformation to acute myeloid leukemia, facilitated by somatic mutation of the receptor for G-CSF.[6] The association between factor use, receptor mutation, and leukemia prompts clinicians to develop better interventions.

As a potential treatment for SCN, Tran et al.[2] provide proof-of-principle for the use of CRISPR/Cas9-mediated gene editing to correct mutations in *ELANE*. The authors selected a mutation in exon 4 of *ELANE* (L172P), which fortuitously creates a protospacer adjacent motif (PAM) sequence required for Cas9 binding and nuclease activity. Taking advantage of the PAM sequence, the authors were able to employ a targeted approach specifically toward the mutated allele. In addition, the authors also employed a "universal" approach, using a sgRNA designed to target exon 4. This universal approach may be more translationally relevant because of the high number of heterogeneous mutations in *ELANE* in the patient population and the absence of a PAM sequence adjacent to the mutation site. Cas9-generated DNA breaks can be corrected by either non-homologous end joining or homologous recombination. To achieve homologous recombination, a wild-type DNA sequence was introduced into the cells using adeno-associated virus serotype 6 (AAV-6). This precision-based approach targeted the mutant allele with high efficiency in patient cells, while the universal approach targeted the wild-type and mutant allele. While >70% efficiency was observed in targeting of the region of interest by either approach, correction of the mutation was observed ∼40% of the time. The results suggest an inherent preference of the cell to utilize homologous recombination over non-homologous end joining repair mechanisms. While repair efficiencies were fairly high, some limitations in the introduction of the repair template still exist.

To test if the observed efficiency of repair in *ELANE* could restore normal granulopoiesis, the authors performed *ex vivo* differentiation of patient hematopoietic stem/progenitor stem cells (HSPCs) that underwent both targeted and universal gene editing. Both approaches demonstrated restoration of differentiation to granulocytes and decreased cell death in repaired cells compared to non-repaired HSPCs. The investigators also showed that neutrophils derived from repaired HSPCs by either approach displayed normal functions. The genetically corrected neutrophils generated reactive oxygen species, phagocytosed, promoted neutrophil extracellular trap formation and gram-negative bacteria killing. However, the study did not address whether ELANE enzymatic activity was normalized. The authors finally demonstrated that the repaired human HSPCs reconstituted granulopoiesis in humanized mice with human neutrophils in the peripheral blood. Inspection of bone marrow demonstrated that both repaired and non-repaired SCN-HSPCs displayed similar engraftment, but only the repaired SCN-HSPCs promoted generation of neutrophil differentiation. A key observation was that the repaired SCN-HSPCs overcame the classical block in differentiation at the promyelocyte stage. Sequencing of early and late stage granulocytic cells showed absence of the mutated allele, confirming that the differentiation was mediated by the repaired HSPCs. The sequencing data further suggest that 40% correction was sufficient to achieve normal granulopoiesis.

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While the authors provided evidence that their gene editing approach did not introduce many off-target effects, the long-term impact remains unknown. It is also unclear whether engraftment of the corrected HSPCs is sustainable. Another unanswered question is whether the risk of transformation to myeloid leukemia is eliminated. Although the authors had the ability to employ specific targeting, the lack of differences between targeted and universal approach is a promising indicator that a more single universal approach can be applied to multiple patients. An alternate approach using CRISPR/Cas9 gene editing is to knock out the *ELANE* gene. Multiple groups have demonstrated that knockout of *ELANE* does not hamper granulopoiesis and that the concurrent knockout of the mutant allele restores differentiation.[7,8] The *ELANE* knockout approach would serve as a universal approach adopted across the wide spectrum of *ELANE* mutations; however, the loss of *ELANE*—a key enzyme for neutrophil function against bacterial and fungal infections—might render patients prone to infections, despite normal neutrophil counts. The adoption of the knockout approach may face other challenges. Tidwell et al.[9] have reported alternate downstream translational start sites for *ELANE* that contribute to SCN pathophysiology. Whether a targeted or universal approach will be used clinically will depend on whether a return to normalcy is achieved. Regardless, CRISPR/Cas9 editing will continue to advance and will herald, at long last, a new age of gene therapy.[10]

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