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Editorial: First Regulatory Approvals for CRISPR-Cas9 Therapeutic Gene Editing for Sickle Cell Disease and Transfusion-Dependent β-Thalassemia

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Abstract In 2020, Emmanuelle Charpentier and Jennifer Doudna were awarded the Nobel Prize in Chemistry for their research on the endonuclease, clustered regularly interspaced short palindromic repeats (CRISPR) and the CRISPR-associated protein 9 (CRISPR-Cas9) method for DNA editing. On 16 November 2023, the UK Medicines and Healthcare Products Regulatory Agency (MHRA) was the first to approve the CRISPR-Cas9 gene editing therapy, Casgevy (exagamglogene autotemcel), for the treatment of patients with transfusion-dependent β-thalassemia and the treatment of sickle cell disease in patients aged ≥12 years with recurrent vaso-occlusive crises. On 8 December 2023, the US Food and Drug Administration (FDA) approved both Casgevy and Lyfgenia (lovotibeglogene autotemcel) for patients with sickle cell disease. On 15 December 2023, the European Medicines Agency (EMA) approved Casgevy for sickle cell disease and transfusion-dependent β-thalassemia. This Editorial aims to present an update on the landmark first regulatory approvals of CRISPR-Cas9 for patients with sickle cell disease and transfusion-dependent β-thalassemia and the potential challenges for therapeutic gene (DNA) editing.

Keywords: **Gene Editing • CRISPR-Cas9 • Sickle Cell Disease • β-Thalassemia • Editorial**

Precise and validated DNA recognition, cleavage, and repair methods are required in gene (DNA) editing [1]. The main approaches for gene modification to treat human disease involve manipulating gene sequences and gene expression [2,3]. Several decades of research on therapeutic gene editing have been hopeful, but ethical issues remain regarding germline gene editing using human oocytes, sperm, or embryos [2,3]. Potential clinical roles for gene editing somatic cells, including hematopoietic stem cells, have also raised safety and ethical concerns [1,2,4].

Therapeutic applications of the three main approaches for therapeutic gene modification have been limited by complications that have halted the initial promise for regulatory approvals [1,2,4]. First, gene therapy for single gene disorders and malignancy has been associated with reports of genotoxicity and immune reactions to gene therapy vectors [2,3]. The second approach of epigenetic gene silencing involves RNA interference (RNAi) by molecular introduction of antisense oligonucleotides (ASOs) or short hairpin RNA (shRNA) that degrade mRNA to reduce the expression of single or multiple genes [2,3]. The third approach to gene modification is gene (DNA) editing, which uses molecular methods to alter established genes involved in human disease [2,3]. Gene editing uses an endonuclease to cut a region of DNA and bring the two cut ends together or insert a new or altered gene sequence [2]. The most studied endonuclease is the clustered regularly interspaced short palindromic repeats (CRISPR) and the CRISPR-associated protein 9 (CRISPR-Cas9) system [5-8].

In 2020, Emmanuelle Charpentier and Jennifer Doudna were awarded the Nobel Prize in Chemistry for their research on the CRISPR-Cas9 method for DNA editing [9,10]. Clustered regularly interspaced short palindromic repeats (CRISPR), initially identified in bacteria to fight bacteriophage infection, also allow bacteria to recognize genetic sequences using specialized enzymes or CRISPR-associated proteins (Cas), including the DNA endonuclease, Cas9 [10]. In 2011, Charpentier and colleagues first identified the trans-activating crRNA (tracrRNA) involved in recognizing foreign phage sequences in bacteria [11]. In 2012, an early collaboration between the Charpentier and Doudna laboratories identified a bacterial CRISPR-Cas9 endonuclease, guided by two RNA molecules forming the tracrRNA: crRNA duplex, which resulted in site-specific cleavage of DNA [12]. It was then possible to manufacture dual tracrRNA: crRNA as a single guide RNA (sgRNA) that retains a 20 nucleotide sequence for site targeting, a double-stranded structure that binds to Cas9 [12]. In 2012, the era of CRISPR-Cas9 genome editing began as gene

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function could be disrupted by CRISPR-Cas9-mediated induction of double-strand DNA breaks and nonhomologous gene repair or gene replacement, and changes in specific bases or insertion of sequences encoding tagged proteins epitopes [9]. The clinical applications result from the possibility for transcriptional activation or repression to control gene expression, for epigenetic alterations, for combination with methyltransferases and acetyltransferases, and multiplex methods to edit multiple genes, and for high-throughput genome-wide screening [13]. The first clinical therapeutic applications for CRISPR-Cas9 began with studies on sickle cell disease and β-thalassemia [1].

There are several clinical subgroups of sickle cell disease (SCD), including hemoglobin SC disease (HbSC), sickle cell anemia (SCA), and hemoglobin sickle-β-thalassemia (β-thalassemia-positive or β-thalassemia-negative), with other minor variants [14,15]. Patients with the sickle cell trait (HbAS) who carry a heterozygous gene mutation do not usually have clinical signs or symptoms [14,15]. Patients with sickle cell anemia suffer from episodes of hemolytic anemia, with bone pain and end-organ damage, and require repeated blood transfusions [14]. The sickle cell mutation has an autosomal dominant inheritance and involves the replacement of negatively charged glutamine by a neutral valine at the sixth position of the β-globin chain, with homozygous mutation resulting in the most severe form of the disease [15]. Other compound heterozygous forms exist, where a single copy of the mutated β-globin gene may be coinherited with another mutated gene [14,15]. For example, in HbSC disease, the sickle cell gene is coinherited with a mutated hemoglobin C (HbC) gene [14,15]. Sickle cell disease and HbAS are more prevalent in sub-Saharan Africa, and West Africa has the highest prevalence of HbSC disease [14]. HbSC accounts for up to 30% of cases of sickle cell disease in the US [16]. In 2023, the US Centers for Disease Control and Prevention (CDC) estimated that approximately 100,000 Americans in total and 1 in 365 African Americans have sickle cell disease [16]. In the US, HbSC disease accounts for 30% of all patients with sickle cell disease [16].

Thalassemia is a common cause of microcytic anemia due to impaired synthesis of hemoglobin globin protein [17]. β-thalassemia is an inherited mutation of the β-globin gene on chromosome 11, causing a reduced β-globin chain of hemoglobin, and occurs most commonly in people of Asian, Mediterranean, and Middle Eastern origin [17,18]. The prevalence of β-thalassemia is between 80-90 million carriers and up to 1.5% of affected individuals in the global population [18]. Patients have varied genotypic and phenotypic presentations because more than 200 mutations have been identified in the β-globin gene [17]. There are three classifications of β-thalassemia: β-thalassemia minor (carrier or trait) is heterozygous and usually asymptomatic with mild anemia; homozygous or compound heterozygous β-thalassemia mutations result in a more severe range of anemias called β-thalassemia intermedia and β-thalassemia major, which are distinguished clinically by transfusion non-dependence or dependence [17,18].

Sickle cell disease and β-thalassemia have become the paradigm for CRISPR-based therapeutic genome editing in human disease, with the first regulatory approvals for Casgevy (exagamglogene autotemcel) and Lyfgenia (lovotibeglogene autotemcel) [19,20]. These gene editing therapies use the patient's hematopoietic stem cells, modified *ex vivo* by genome editing using CRISPR-Cas9 technology, and then transplanted as a single-dose infusion [19,20]. Before treatment, the patient's stem cells are collected and undergo myeloablative conditioning with high-dose chemotherapy. The modified stem cells are then transplanted to the patient, engrafting within the bone marrow [19,20]. Casgevy (exagamglogene autotemcel) is used to edit $CD34^+$ cell human hematopoietic stem and progenitor cells at the erythroid-specific enhancer region of the BCL11A gene, which prevents the production of fetal hemoglobin (HbF) [21]. Lyfgenia (lovotibeglogene autotemcel) is a cell-based gene therapy that uses a lentiviral vector with blood stem cells genetically modified to produce HbAT87Q, which functions in a similar way to normal adult hemoglobin (HbA) to reduce the risk of red blood cell sickling and to improve vascular blood flow [21].

On 16 November 2023, the UK Medicines and Healthcare products Regulatory Agency (MHRA) was the first regulatory agency to approve the CRISPR-Cas9 gene editing therapy, Casgevy (exagamglogene autotemcel), for the treatment of patients aged ≥12 years with transfusion-dependent β-thalassemia for whom hematopoietic stem cell transplantation is appropriate and a human leukocyte antigen (HLA)-matched related hematopoietic stem cell donor is not available [21,22]. Also, on 16 November 2023, Casgevy (exagamglogene autotemcel) was approved in the UK by the MHRA for the treatment of sickle cell disease in patients aged ≥12 years with recurrent vaso-occlusive crises, and for whom hematopoietic stem cell transplantation is appropriate and an HLA-matched related hematopoietic stem cell donor is not available [22].

The studies that supported the approval of Casgevy (exagamglogene autotemcel) include ongoing, single-arm, multicenter safety and efficacy studies in adolescents and adults with sickle cell disease and β-thalassemia [22]. Patients with sickle cell disease were included with a history of at least two severe episodes of vaso-occlusive disease during each of the two years before screening [22]. The primary efficacy outcome measure was freedom from severe vaso-occlusive disease for at least 12 consecutive months during the 24-month follow-up period [22]. There were 31 patients treated with Casgevy (exagamglogene autotemcel) with sufficient follow-up time, of which 29 patients (93.5%) achieved the primary efficacy outcome [22]. However, all 31 treated patients achieved successful cell engraftment

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with no cases of graft rejection or failure [22]. The most commonly reported side effects of Casgevy (exagamglogene autotemcel) were reduced platelet and white blood cell (WBC) levels, nausea, mouth ulcers, musculoskeletal and abdominal pain, and febrile neutropenia [22].

The results from a single-arm 24-month multicenter study in 32 patients with sickle cell disease and a history of vaso-occlusive disease aged between 12-50 years supported the initial safety and efficacy of another CRISPR-Cas9 gene editing therapy, Lyfgenia (lovotibeglogene autotemcel) [22]. The efficacy of Lyfgenia (lovotibeglogene autotemcel) was determined by the complete resolution of vaso-occlusive events between 6-18 months after infusion with Lyfgenia (lovotibeglogene autotemcel) [22]. Twenty-eight (88%) patients achieved complete resolution of vaso-occlusive events during the study [22]. The most common side effects of Lyfgenia (lovotibeglogene autotemcel) included stomatitis, reduced platelet and WBC levels, and febrile neutropenia [22]. Of concern is that there have been reports of hematologic malignancy in patients treated with Lyfgenia (lovotibeglogene autotemcel), resulting in a black box warning of this risk, with a recommendation that treated patients should have long-term follow-up [22].

On 8 December 2023, the US Food and Drug Administration (FDA) approved Casgevy (exagamglogene autotemcel) and Lyfgenia (lovotibeglogene autotemcel) under the FDA's Priority Review, Orphan Drug, Fast Track, and Regenerative Medicine Advanced Therapy designations [23]. The FDA approval for Casgevy (exagamglogene autotemcel) was for the treatment of sickle cell disease in patients aged ≥12 years with recurrent vaso-occlusive crises [23]. On 15 December 2023, the European Medicines Agency (EMA) approved Casgevy (exagamglogene autotemcel) for both sickle cell disease and transfusion-dependent β-thalassemia [24]. On 16 January 2024, the FDA approved a Biologics License Application (BLA) for Casgevy (exagamglogene autotemcel) for transfusion-dependent β-thalassemia [25].

Recent developments in therapeutic CRISPR-Cas9 DNA editing are significant in molecular-targeted therapeutics [26]. However, because of safety concerns regarding editing DNA, developments in RNA-editing therapies are receiving attention [4, 27]. In February 2024, RNA-editing treatments for two genetic diseases, alpha-1 antitrypsin deficiency (AATD) and Stargardt disease, a form of juvenile macular dystrophy, have recently gained approval for clinical trials [27]. RNA-editing therapies raise hopes for safer treatments because, unlike CRISPR genome editing, RNA editing does not alter genes but single bases to produce deficient proteins [27]. One RNA-editing approach is single-base editing, which utilizes the cell enzyme adenosine deaminase acting on RNA (ADAR), which replaces adenine for inosine in the RNA sequence [27]. A second approach is to use RNA exon editing to change multiple mutations in the genome [27]. RNA-based therapies may treat malignancy by generating proteins that inhibit tumor cell growth or replacing an RNA sequence associated with a tumor growth factor [27].

Conclusions

The first regulatory approvals of therapeutic CRISPR-Cas9 for patients with sickle cell disease and transfusion-dependent β-thalassemia mark the beginning of the long-awaited realization of the promise of therapeutic gene (DNA) editing. Currently, patients with sickle cell disease and β-thalassemia who receive Casgevy (exagamglogene autotemcel) and Lyfgenia (lovotibeglogene autotemcel) are undergoing long-term evaluation on the safety and effectiveness of these CRISPR-Cas9 DNA editing therapies. Recognizing potential safety concerns associated with gene editing highlights the importance of patient monitoring during and after CRISPR-Cas9 treatment. Recent studies on RNA-editing therapies bring further hopes for targeted therapeutic molecular approaches to modify cell proteins without the potential safety concerns associated with gene (DNA) editing.

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