Gene Editing in Hematology (Casgevy, Lyfgenia)

Introduction :

Hemoglobinopathies, including sickle cell disease (SCD) and β-thalassemia, are common monogenic disorders affecting millions of people worldwide. The World Health Organization estimates that over 5% of the global population carries hemoglobinopathies, with nearly 300,000 – 400,000 children born annually with severe forms of these diseases (1, 2). Hemoglobinopathies are caused by perturbed hemoglobin (Hb) biosynthesis, affecting the structure, function or production of Hb which is the oxygen-carrying protein in red blood cells (RBCs). The normal adult Hb molecule, known as Hemoglobin A (HbA), Is composed of two α-globin chains and two β-globin chains (3). Hemoglobinopathies are broadly classified into two subgroups: a) Hemoglobinopathies with Hb structural aberrations that include SCD, Hemoglobin C (HbC), Hemoglobin D (HbD), Hemoglobin E (HbE), and Hemoglobin Constant Spring disease (HbCS), and b) Hemoglobinopathies with abnormal Hb production include thalassemia syndrome (α− and β−thalassemia). SCD is caused by a point mutation in β-globin (HBB) gene that substitutes Valine for Glutamic acid at codon 6 (βG6V). G6V mutation results in production of sickle hemoglobin (HbS) which forms long polymers under poor oxygenation. HbS polymerization distorts the shape of RBC with a characteristic sickle shape (4). SCD syndrome is characterized by hemolysis, vaso-occlusion crises (VOC), and infections causing recurring episodes of acute pain, tissue damage, strokes, and potentially resulting in multi-organ damage or death in most severe cases (5). β-thalassemias are caused by mutations in HBB gene resulting in absence (beta-zero (β0) Thalassemia) or quantitative reduction (beta-plus (β+) thalassemia) in β-globin production (6). With a reduction In β-globin, the formation of functional Hb complex is impaired and normal development of RBC is affected, leading to anemia and associated health complications. β-thalassemia is caused by more than 200 mutations in the HBB gene and are classified in to major, intermedia, and minor forms depending on clinical severities with symptoms like severe anemia, bone deformities, and organ damage (7).

Literature Review :

1-A Historical Overview of Sickle Cell Therapy: From Stem Cell Transplantation to Gene Therapy:

Until recently, treatment options for SCD and β-thalassemia were primarily limited to palliative symptomatic managements and allogeneic hematopoietic stem cell transplant (HSCT). However, challenges like unavailability of compatible donors and immunological risks associated with allogeneic HSCT necessitate more effective solutions. As the root cause of SCD and β-thalassemia lies in mutations in the HBB gene, employing genetic approaches targeting the mutant HBB gene present the most promising and effective approach. In 2023, the U.S. Food and Drug Administration (FDA) granted approval to two groundbreaking therapies for SCD patients: CASGEVY (exagamglogene autotemcel) and LYFGENIA (lovotibeglogene autotemcel). The approval of two SCD gene therapies represents a significant advancement, showcasing the role of innovative technologies in transforming medical treatment (7).

2-Clinical Translation of CRISPR/Cas9: Advances, Applications, and Challenges :

In recent years, clustered regularly interspaced short palindromic repeats (CRISPRs) and CRISPR-associated (Cas) proteins have emerged as revolutionary gene editing tools to treat Inherited disorders affecting different organ systems, such as blood and muscles. Both hematological and neuromuscular genetic disorders benefit from genome editing approaches but face different challenges in their clinical translation. The ability of CRISPR/Cas9 technologies to modify hematopoietic stem cells ex vivo has greatly accelerated the development of genetic therapies for blood disorders(8) .

In the last decade, many clinical trials have been initiated, delivering encouraging results. The recent FDA approval of Casgevy, the first CRISPR/Cas9-based therapy for severe sickle cell disease and transfusion-dependent β-thalassemia, represents a significant milestone in the field and highlights the great potential of this technology. Similar preclinical efforts are currently expanding CRISPR therapies to other hematologic disorders, such as primary immunodeficiencies (8).

In the neuromuscular field, the versatility of CRISPR/Cas9 has been instrumental for the generation of new cellular and animal models of Duchenne muscular dystrophy (DMD), offering innovative platforms to speed up preclinical development of therapeutic solutions. Several corrective interventions have been proposed to genetically restore dystrophin production using the CRISPR toolbox and have demonstrated promising results in different DMD animal models. Although these

advances represent a significant step forward in the clinical translation of CRISPR/Cas9 therapies for DMD, there are still many hurdles to overcome, such as in vivo delivery methods associated with high viral vector doses, together with safety and immunological concerns (8).

Collectively, the results obtained in the hematological and neuromuscular fields emphasize the transformative impact of CRISPR/Cas9 for patients affected by these debilitating conditions. As each field suffers from different and specific challenges, the clinical translation of CRISPR therapies may progress differently depending on the genetic disorder. Ongoing investigations and clinical trials will continue to address risks and limitations of these therapies, including long-term efficacy, potential genotoxicity, and adverse immune reactions (8).

The CRISPR/Cas9 system uses a chimeric single-guide RNA (sgRNA) to direct an endonuclease (Cas9) to specific DNA targets, where it generates double-strand breaks (DSBs)(9).These DSBs can be repaired by the cell through two competing pathways: the error-prone non-homologous end joining, which generates insertions and deletions at the cutting site, or the more precise homology-directed repair, which requires a DNA template to introduce specific modifications(10).Pioneering CRISPR/Cas9 approaches have been successfully used in several clinical trials for lung cancer (11) and for β-hemoglobinopathies Despite these successes, it is becoming more evident that Cas9-induced DSBs can lead to genotoxic effects that include large insertions/deletions, chromosomal translocation, and chromothripsis [12,13,14,15], raising long-term safety concerns .

3- FDA-Approved Gene Therapies for Sickle Cell Disease and β-Thalassemia: Casgevy and Lyfgenia

Both Casgevy (exagamglogene autotemcel) and Lyfgenia (lovotibeglogene autotemcel) are autologous hematopoietic stem cell-based gene therapies.(16, 17, 18) On December 8, 2023, under Priority Review and Fast Track designation, each therapy received FDA approval for individuals aged 12 years and older with SCD who experience recurrent VOCs.(16) Additionally, on January 16, 2024, Casgevy received FDA approval for individuals aged 12 years and older who have transfusion-dependent β-thalassemia(19) .

The products reduce red blood cell sickling by different mechanisms. Casgevy utilizes CRISPR/Cas9 technology for gene editing to downregulate BCL11A, whichh inhibits the transition from fetal hemoglobin (HbF) to adult hemoglobin.(17,18) HbF has a higher affinity for oxygen than HbS and inhibits the polymerization of HbS(17, 18) .

In contrast, Lyfgenia is a lentiviral vector that expresses a novel HbAT87Q variant of hemoglobin, which functions similarly to normal adult hemoglobin (HbA)(16,20) .

In the ongoing clinical trial (NCT03745287), the interim efficacy of Casgevy was evaluated. Out of the 44 participants who received Casgevy, 30 had at least 12 consecutive months of follow-up, with 29 individuals (97%) achieving freedom from severe VOCs.(16,17)Notably, all treated individuals experienced successful engraftment without any cases of graft failure or rejection.(16,17)

As per the interim analysis from the Phase 1/2 HGB-206 clinical trial (NCT02140554), 28 out of 32 (87.5%)participants who received Lyfgenia achieved freedom from VOCs during follow-up, which ranged from 6 to 18 months after treatment (16,21).

Common adverse effects of Casgevy include leukopenia, thrombocytopenia, neutropenic fever, mouth sores, nausea, vomiting, abdominal pain, musculoskeletal pain, headache, and itching.16 Similarly, common adverse effects of Lyfgenia include stomatitis, leukopenia, anemia, thrombocytopenia, and febrile neutropenia.16 These adverse effects were generally consistent with the use of myeloablative busulfan conditioning and autologous HSCT. Acute myeloid leukemia has occurred in individuals treated with Lyfgenia, and the FDA has added a boxed warning on the label with information regarding this risk.(16,22) Casgevy does not contain any boxed warnings for prescribers .

Table 1.

Comparisons of the 2 gene therapies for sickle cell disease

Feature	Casgevy (Exagamglogene Autotemcel)	Lyfgenia (Lovotibeglogene Autotemcel)
Mechanisms	A cell-based gene therapy using CRISPR/Cas9	A cell-based gene therapy utilizing a lentiviral vector as a gene delivery vehicle for genetic modification
Effects	By silencing erythroid-specific *BCL11A* enhancer, the goal is to increase the production of HbF	By delivering engineered hemoglobin containing missense HBB 87T>Q variant which has anti-sickling properties similar to HbF
Common grounds	Individuals' hematopoietic stem cells are collected and genetically modified to prepare them for treatment. These stem cells are reinfused after high-dose chemotherapy.	
Adverse effects	Leukopenia, thrombocytopenia, neutropenic fever, mouth sores, nausea, vomiting, abdominal pain, musculoskeletal pain, headache, and itching	Stomatitis, leukopenia, anemia, thrombocytopenia, and febrile neutropenia. One individual developed acute myeloid leukemia after treatment.
Comments	The first FDA-approved gene therapy utilizing CRISPR/Cas9, a type of genome editing technology. This approval marked the integration of CRISPR/Cas9 into clinical practice.	Individuals who receive Lyfgenia may be at risk of developing hematologic malignancies. Therefore, it is recommended that these patients undergo lifelong monitoring.

FDA, Food and Drug Administration; *HbF*, fetal hemoglobin.

Discussion

Casgevy and Lyfgenia have been hailed as breakthroughs in the field of medicine, providing a potential cure for hundreds of thousands of patients affected by SCD. Both therapies have been approved by the FDA after showing efficacy in separate clinical trials. Until now, no effective treatment has been discovered for SCD except bone marrow transplantation, which contains its own set of risks. Both Casgevy and Lyfgenia hold promising futures for curing SCD in the United States and potentially worldwide. These advancements are poised to accelerate research and development in gene therapy, fostering a burgeoning field dedicated to enhancing treatment outcomes and quality of life for patients with genetic disorders worldwide. This noteworthy development underscores the promise of cell-based therapies as a viable alternative to currently prescribed standard drugs .

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