β-Hemoglobinopathies

Subjects: Oncology

Contributor: Annarita Miccio

β-hemoglobinopathies are the most common genetic disorders worldwide and are caused by mutations affecting the production or the structure of adult hemoglobin. Patients affected by these diseases suffer from anemia, impaired oxygen delivery to tissues, and multi-organ damage. In the absence of a compatible donor for allogeneic bone marrow transplantation, the lifelong therapeutic options are symptomatic care, red blood cell transfusions and pharmacological treatments. The last decades of research established lentiviral-mediated gene therapy as an efficacious therapeutic strategy. However, this approach is highly expensive and associated with a variable outcome depending on the effectiveness of the viral vector and the quality of the cell product. In the last years, genome editing emerged as a valuable tool for the development of curative strategies for β-hemoglobinopathies. Moreover, due to the wide range of its applications, genome editing has been extensively used to study regulatory mechanisms underlying globin gene regulation allowing the identification of novel genetic and pharmacological targets.

genome editing β-hemoglobinopathies

1. Introduction

Hemoglobinopathies are the most frequent monogenic diseases worldwide, with approximately 400,000 affected births each year [1]. The most common hemoglobinopathies are β-thalassemia and sickle cell disease (SCD). Patient affected by β-thalassemia show low or absent production of adult β-globin chains; this leads to α-/β-globin chain imbalance, apoptosis of erythroid cells, hemolysis and iron overload. In SCD, a single point mutation causes the $\beta 7^{Glu \rightarrow Val}$ substitution that leads to the production of a mutant β-globin chain (β^S). Once incorporated in the hemoglobin tetramer (HbS), the valine in position 7 confers to the Hb the propensity to polymerize. HbS polymerization causes red blood cell (RBC) sickling, hemolysis, iron overload, vaso-occlusive crises and multi-organ damage.

Palliative treatment consisting of lifetime blood transfusion combined with iron chelation, although costly, is commonly used to suppress anemia and reduce iron toxicity in β-thalassemia patients. SCD patients with a severe clinical phenotype are usually treated with RBC exchange transfusion and iron chelation. In developed countries, this standard treatment for β-hemoglobinopathies improves patients' quality of life. However, these diseases can still result in fatal outcomes. In African countries, where SCD is endemic, the majority of children born with SCD do not reach adulthood [1]. Allogeneic hematopoietic stem cell transplantation (HSCT) is a curative option, but its success depends on the availability of a compatible human leukocyte antigen (HLA)-matched donor. Studies in large cohorts of β-thalassemia and SCD patients receiving identical sibling transplants showed 83% and 91% of

disease-free survival, respectively, with better clinical outcomes in younger patients [2][3]. However, the chances of finding an HLA-matched sibling or related donor are limited [4]. Recently, it has been shown that unrelated HLA-matched transplantation is possible for β-thalassemia patients lacking of an HLA-matched sibling donor, when stringent criteria of HLA class I and II compatibility between the donor and the recipient are met. Despite the limited number of reported cases, results in β-thalassemia patients are encouraging, thus this approach represents a promising therapeutic option [5][6].

Gene therapy nowadays represents an important curative option for β-hemoglobinopathies. The transplantation of genetically modified, autologous HSCs avoids the immunological risks such as graft rejection and graft-versus-host disease associated with allogeneic HSCT and use of immunosuppressive drugs to prevent these complications. In the last decades, several research groups engineered lentiviral vectors (LV) to successfully transfer a functional β-globin chain in HSC-derived erythroid cells [7][8][9][10]. These paramount studies paved the way for several gene therapy clinical trials including more than 100 patients treated with LV-transduced bone marrow (BM)- or mobilized peripheral blood (mPB)-derived HSCs [11][12][13][14][15][16][17][18][19][20][21][22]. The majority of these patients showed a reduced transfusion requirement after gene therapy and this treatment can be considered reasonably safe. However, some patients, especially those with a more severe clinical phenotype, are still transfusion-dependent, because the optimal therapeutic Hb levels have not been achieved and/or because of the low gene transfer in HSCs. Current progresses and challenges of LV-mediated gene therapy approaches for β-hemoglobinopathies are reviewed elsewhere [23]. Briefly, the major limitations are: (i) the lack of an optimal protocol to reproducibly obtain a successful gene transfer in HSCs; (ii) the inability of LVs to sustain high β-globin expression at low vector copy numbers; (iii) the poor engraftment of transduced HSCs into the patient BM environment, which is still poorly studied and negatively affected by disease progression; (iv) the potential genotoxic risk associated with the semi-random integration of LVs [24].

In the last decade, the numerous discoveries in the field of genome editing have led to the development of novel and powerful strategies for the treatment of β-hemoglobinopathies. In particular, the efficiency of genome editing was greatly enhanced by the development of designer nucleases to generate DNA double strand breaks (DSBs) in a specific region of interest. Different nuclease-based editing strategies aimed either to correct the disease-causing mutations or to induce therapeutic levels of fetal Hb (HbF) have been proposed.

The success of these strategies depends on the efficacy of the nucleases to target the DNA, the activity of the approach-specific cellular machinery repairing the DSB in HSCs and the capability of edited cells to stably engraft and provide high levels of therapeutic Hb. However, nucleases can trigger DSB-induced cytotoxicity and the formation of chromosomal rearrangements. The recent introduction of nuclease-free technologies has provided suitable solutions to overcome these issues and is likely shortening the way for the clinical translation of editing strategies for the treatment of β-hemoglobinopathies.

2. Genome Editing for β-Hemoglobinopathies

Genome editing technologies provided a fertile soil for the development of novel therapeutic strategies for β-hemoglobinopathies. The last years have witnessed the development of an increasing number of technologies, experimental models and genetic targets for genome editing-based therapies. The study of cellular mechanisms allowing gene correction and regulatory mechanisms underlying Hb switching opened the way for the preclinical and clinical application of novel gene therapy approaches, which could become in the future a widespread therapeutic option for the high number of patients affected by β-hemoglobinopathies.

However, while many therapeutic targets have been identified and, for some strategies, early clinical data are very encouraging [25][26][27], the genome editing technologies still face some challenges. A first risk is the off-target effects on DNA and on both DNA and RNA in the case of BEs. The transient expression of genome editing tools and the use of more precise enzymes can substantially reduce off-target effects [28][29]. However, even minimal off-target activity needs to be closely monitored in both pre-clinical and clinical studies, as gene therapy approaches are based on the injection of a large number of HSPCs.

Similarly, HSPC viability, proliferation, engraftment and genome integrity must be carefully evaluated especially when using nuclease-based strategies that can affect cellular fitness and functionality [36], and generate large genomic rearrangements [30][31][32]. However, amelioration of culture conditions and delivery methods (e.g., usage of ssODN instead of AAV6 as donor template), and the use of highly specific editing tools can substantially reduce cytotoxicity [33] and the formation of genomic rearrangements, such as translocations between on- and off-target regions.

The novel BE and PE tools virtually abolish the risk of DSB-induced cytotoxicity and large rearrangements. However, while HSPC electroporation with nucleases has been established in many labs and is already employed in clinical trials, delivery of the large BE and PE enzymes is still challenging in primary cells.

Finally, pre-existing immunity against Cas9 or de novo immune responses to Cas9 should be taken in consideration; however, the transient expression of the CRISPR/Cas9-based tools likely minimizes the risk of elimination of genetically modified HSPCs by the immune system.

Despite these challenges, genome editing approaches hold promise for curing patients affected β-hemoglobinopathies. Nowadays, LV-mediated gene therapy strategies for β-hemoglobinopathies represent an established curative option. However, it is important to note that LVs are extremely expensive to manufacture (>300,000 euros per patient [34]). Most of the genome-editing approaches require the delivery of RNA/protein reagents that are likely less expensive thus, it is reasonable to suppose that they will replace LV-based gene therapy strategies in the coming years.

References

1. Piel, F.B.; Patil, A.P.; Howes, E.R.; Nyangiri, A.O.; Gething, P.W.; Dewi, M.; Temperley, W.H.; Williams, T.N.; Weatherall, D.J.; Hay, I.S. Global epidemiology of sickle haemoglobin in neonates: A contemporary geostatistical model-based map and population estimates. Lancet 2013, 381, 142–151.
2. Angelucci, E.; Matthes-Martin, S.; Baronciani, D.; Bernaudin, F.; Bonanomi, S.; Cappellini, M.D.; Dalle, J.-H.; Di Bartolomeo, P.; De Heredia, C.D.; Dickerhoff, R.; et al. Hematopoietic stem cell transplantation in thalassemia major and sickle cell disease: Indications and management recommendations from an international expert panel. Haematologica 2014, 99, 811–820.
3. Gluckman, E.; Cappelli, B.; Bernaudin, F.; Labopin, M.; Volt, F.; Carreras, J.; Simões, B.P.; Ferster, A.; Dupont, S.; De La Fuente, J.; et al. Sickle cell disease: An international survey of results of HLA-identical sibling hematopoietic stem cell transplantation. Blood 2017, 129, 1548–1556.
4. Lucarelli, G.; Andreani, M.; Angelucci, E. The cure of thalassemia by bone marrow transplantation. Blood Rev. 2002, 16, 81–85.
5. Locatelli, F.; Merli, P.; Strocchio, L. Transplantation for thalassemia major. Curr. Opin. Hematol. 2016, 23, 515–523.
6. Strocchio, L.; Locatelli, F. Hematopoietic Stem Cell Transplantation in Thalassemia. Hematol. Clin. N. Am. 2018, 32, 317–328.
7. May, C.; Rivella, S.; Callegari, J.; Heller, G.; Gaensler, K.M.L.; Luzzatto, L.; Sadelain, M. Therapeutic haemoglobin synthesis in β-thalassaemic mice expressing lentivirus-encoded human β-globin. Nature 2000, 406, 82–86.
8. Pawliuk, R.; Westerman, K.A.; Fabry, M.E.; Payen, E.; Tighe, R.; Bouhassira, E.E.; Acharya, S.A.; Ellis, J.; London, I.M.; Eaves, C.J.; et al. Correction of Sickle Cell Disease in Transgenic Mouse Models by Gene Therapy. Science 2001, 294, 2368–2371.
9. Rivella, S.; May, C.; Chadburn, A.; Riviere, I.; Sadelain, M. A novel murine model of Cooley anemia and its rescue by lentiviral-mediated human β-globin gene transfer. Blood 2003, 101, 2932–2939.
10. Miccio, A.; Cesari, R.; Lotti, F.; Rossi, C.; Sanvito, F.; Ponzoni, M.; Routledge, S.J.E.; Chow, C.-M.; Antoniou, M.N.; Ferrari, G. In vivo selection of genetically modified erythroblastic progenitors leads to long-term correction of -thalassemia. Proc. Natl. Acad. Sci. USA 2008, 105, 10547–10552.
11. Cavazzana-Calvo, M.; Payen, E.; Negre, O.; Wang, G.; Hehir, K.; Fusil, F.; Down, J.; Denaro, M.; Brady, T.; Westerman, A.K.; et al. Transfusion independence and HMGA2 activation after gene therapy of human β-thalassaemia. Nature 2010, 467, 318–322.

12. Kanter, J.; Walters, M.C.; Hsieh, M.; Krishnamurti, L.; Kwiatkowski, J.L.; Kamble, R.; von Kalle, C.; Joseney-Antoine, M.; Pierciey, F.J.; Shi, W.; et al. Interim Results from a Phase 1/2 Clinical Study of Lentiglobin Gene Therapy for Severe Sickle Cell Disease. Blood 2017, 130, 527.
13. Kwiatkowski, J.L.; Thompson, A.A.; Rasko, J.; Hongeng, S.; Schiller, G.J.; Anurathapan, U.; Cavazzana, M.; Ho, P.J.; von Kalle, C.; Kletzel, M.; et al. Clinical Outcomes up to 3 Years Following Lentiglobin Gene Therapy for Transfusion-Dependent β-Thalassemia in the Northstar Hgb-204 Study. Blood 2017, 130, 360.
14. Locatelli, F.; Walters, M.C.; Kwiatkowski, J.L.; Porter, J.; Sauer, M.G.; Thuret, I.; Hongeng, S.; Kulozik, A.E.; Lal, A.; Thrasher, A.J.; et al. Lentiglobin Gene Therapy for Patients with Transfusion-Dependent β-Thalassemia (TDT): Results from the Phase 3 Northstar-2 and Northstar-3 Studies. Blood 2018, 132, 1025.
15. Malik, P.; Grimley, M.; Quinn, C.T.; Shova, A.; Courtney, L.; Lutzko, C.; Kalfa, T.A.; Niss, O.; Mehta, A.P.; Chandra, S.; et al. Gene Therapy for Sickle Cell Anemia Using a Modified Gamma Globin Lentivirus Vector and Reduced Intensity Conditioning Transplant Shows Promising Correction of the Disease Phenotype. Blood 2018, 132, 1021.
16. Mansilla-Soto, J.; Riviere, I.; Boulad, F.; Sadelain, M. Cell and Gene Therapy for the Beta-Thalassemias: Advances and Prospects. Hum. Gene Ther. 2016, 27, 295–304.
17. Marktel, S.; Scaramuzza, S.; Cicalese, M.P.; Giglio, F.; Galimberti, S.; Lidonnici, M.R.; Calbi, V.; Assanelli, A.; Bernardo, M.E.; Rossi, C.; et al. Intrabone hematopoietic stem cell gene therapy for adult and pediatric patients affected by transfusion-dependent β-thalassemia. Nat. Med. 2019, 25, 234–241.
18. Ribeil, J.-A.; Hacein-Bey-Abina, S.; Payen, E.; Magnani, A.; Semeraro, M.; Magrin, E.; Caccavelli, L.; Neven, B.; Bourget, P.; El Nemer, W.; et al. Gene Therapy in a Patient with Sickle Cell Disease. N. Engl. J. Med. 2017, 376, 848–855.
19. Sii-Felice, K.; Giorgi, M.; Leboulch, P.; Payen, E. Hemoglobin disorders: Lentiviral gene therapy in the starting blocks to enter clinical practice. Exp. Hematol. 2018, 64, 12–32.
20. Thompson, A.A.; Walters, M.C.; Kwiatkowski, J.; Rasko, J.E.; Ribeil, J.-A.; Hongeng, S.; Magrin, E.; Schiller, G.J.; Payen, E.; Semeraro, M.; et al. Gene Therapy in Patients with Transfusion-Dependent β-Thalassemia. N. Engl. J. Med. 2018, 378, 1479–1493.
21. Tisdale, J.F.; Kanter, J.; Mapara, M.Y.; Kwiatkowski, J.L.; Krishnamurti, L.; Schmidt, M.; Miller, A.L.; Pierciey, J.F.J.; Shi, W.; Ribeil, J.-A.; et al. Current Results of Lentiglobin Gene Therapy in Patients with Severe Sickle Cell Disease Treated Under a Refined Protocol in the Phase 1 Hgb-206 Study. Blood 2018, 132, 1026.
22. Walters, M.C.; Hongeng, S.; Kwiatkowski, J.L.; Locatelli, F.; Porter, J.B.; Sauer, M.G.; Thrasher, A.J.; Thuret, I.; Yannaki, E.; Elliot, H.; et al. Results from the Hgb-207 (Northstar-2) Trial: A Phase

3 Study to Evaluate Safety and Efficacy of Lentiglobin Gene Therapy for Transfusion-Dependent β-Thalassemia (TDT) in Patients with Non-β0/β0 Genotypes. Blood 2017, 130, 526.

23. Magrin, E.; Miccio, A.; Cavazzana, M. Lentiviral and genome-editing strategies for the treatment of β-hemoglobinopathies. Blood 2019, 134, 1203–1213.
24. Ghiaccio, V.; Chappell, M.; Rivella, S.; Breda, L. Gene Therapy for Beta-Hemoglobinopathies: Milestones, New Therapies and Challenges. Mol. Diagn. Ther. 2019, 23, 173–186.
25. Esrick, E.B.; Lehmann, L.E.; Biffi, A.; Achebe, M.; Brendel, C.; Ciuculescu, M.F.; Daley, H.; MacKinnon, B.; Morris, E.; Federico, A.; et al. Post-Transcriptional Genetic Silencing of BCL11A to Treat Sickle Cell Disease. N. Engl. J. Med. 2021, 384, 205–215.
26. Frangoul, H.; Altshuler, D.; Cappellini, M.D.; Chen, Y.-S.; Domm, J.; Eustace, B.K.; Foell, J.; De La Fuente, J.; Grupp, S.; Handgretinger, R.; et al. CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β-Thalassemia. N. Engl. J. Med. 2021, 384, 252–260.
27. Frangoul, H.; Bobruff, Y.; Cappellini, M.D.; Corbacioglu, S.; Fernandez, R.C.M.; De La Fuente, J.; Grupp, A.S.; Handgretinger, R.; Ho, T.W.; Imren, S.; et al. Safety and Efficacy of CTX001 in Patients with Transfusion-Dependent β-Thalassemia and Sickle Cell Disease: Early Results from the Climb THAL-111 and Climb SCD-121 Studies of Autologous CRISPR-CAS9-Modified CD34+ Hematopoietic Stem and Progenitor Cells. Blood 2020, 136, 3–4.
28. Gehrke, J.M.; Cervantes, O.; Clement, K.; Wu, Y.; Zeng, J.; Bauer, D.E.; Pinello, L.; Joung, J.K. An APOBEC3A-Cas9 base editor with minimized bystander and off-target activities. Nat. Biotechnol. 2018, 36, 977–982.
29. Gaudelli, N.M.; Lam, D.K.; Rees, H.A.; Solá-Esteves, N.M.; Barrera, L.A.; Born, D.A.; Edwards, A.; Gehrke, J.M.; Lee, S.-J.; Liquori, A.J.; et al. Directed evolution of adenine base editors with increased activity and therapeutic application. Nat. Biotechnol. 2020, 38, 892–900.
30. Kosicki, M.; Tomberg, K.; Bradley, A. Repair of double-strand breaks induced by CRISPR–Cas9 leads to large deletions and complex rearrangements. Nat. Biotechnol. 2018, 36, 765–771.
31. Cullot, G.; Boutin, J.; Toutain, J.; Prat, F.; Pennamen, P.; Rooryck, C.; Teichmann, M.; Rousseau, E.; Lamrissi-Garcia, I.; Guyonnet-Duperat, V.; et al. CRISPR-Cas9 genome editing induces megabase-scale chromosomal truncations. Nat. Commun. 2019, 10, 1–14.
32. Turchiano, G.; Andrieux, G.; Blattner, G.; Pennucci, V.; Klermund, J.; Monaco, G.; Poddar, S.; Mussolino, C.; Cornu, T.I.; Boerries, M.; et al. Quantitative Evaluation of Chromosomal Rearrangements in Primary Gene-Edited Human Stem Cells by Preclinical CAST-Seq. Cell Stem Cell 2020.
33. Schiroli, G.; Conti, A.; Ferrari, S.; Della Volpe, L.; Jacob, A.; Albano, L.; Beretta, S.; Calabria, A.; Vavassori, V.; Gasparini, P.; et al. Precise Gene Editing Preserves Hematopoietic Stem Cell

Function following Transient p53-Mediated DNA Damage Response. Cell Stem Cell 2019, 24, 551–565.

34. Coquerelle, S.; Ghardallou, M.; Rais, S.; Taupin, P.; Touzot, F.; Boquet, L.; Blanche, S.; Benaouadi, S.; Brice, T.; Tuchmann-Durand, C.; et al. Innovative Curative Treatment of Beta Thalassemia: Cost-Efficacy Analysis of Gene Therapy Versus Allogenic Hematopoietic Stem-Cell Transplantation. Hum. Gene Ther. 2019, 30, 753–761.