

Review Article

Post ASH 2022: Hemoglobinopathies

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ABSTRACT:

In keeping with past years, the 64th annual hybrid meeting of the American Society of Hematology (ASH) provided us with valuable updated information on current clinical research in all major fields of hematology, including hemoglobinopathies. The aim of the present article is to summarize the most recent and promising data in hemoglobinopathies, especially regarding new treatment options. Most of the presentations and posters concerned sickle cell disease, which is the most prevalent hemoglobin disorder in the United States. Efforts are underway to improve the quality of life of patients with hemoglobin disorders and identify and eliminate all these factors that increase mortality and morbidity. Thalassemia gene therapy and sickle cell gene editing are showing promising results over the long term. Research has also been focused on developing novel drugs such as luspatercept in β -thalassemia and inhibitors of the complement system cascade in sickle cell disease. Finally, the presence of clonal hematopoiesis of Indeterminate Potential and its relationship with complications development has been investigated in patients with β -thalassemia.

KEY WORDS: Hemoglobinopathy, sickle cell disease, thalassemia, ASH annual meeting

In keeping with past years, the 64th annual hybrid meeting of the American Society of Hematology (ASH) provided us with valuable updated information on current clinical research in all major fields of hematology, including hemoglobinopathies. The present article summarizes the most promising data in hemoglobinopathies, especially regarding new treatment options. Sickle cell disease is the most prevalent hemoglobin disorder in the USA. This topic was the focus of the most interesting oral presentations and posters on hemoglobinopathies.

Currently, the only cure for hemoglobinopathies is hemopoietic stem cell transplantation, limited by the paucity of suitable donors, and it is only available in high-income countries. Conversely, patients with hemoglobinopathies have had better and safer supportive care,

such as red blood cell transfusions, chelation therapy, drugs modulating hemoglobin E, and vaccinations. Although many efforts are underway to improve quality of life, unmet needs remain. Thalassemia gene therapy and sickle cell gene editing are showing promising results over the long term.

Developing gene transfer for thalassemia has been a long and arduous process. Hematopoietic stem cells are expected to be the target of gene transfer. Hematopoietic stem cells can be gene-transferred with a variety of vectors, but retroviruses are the leading candidate. As part of beti- cel ex vivo gene therapy, the modified HBB gene

Abbreviations: ASH: American Society of Hematology, TDT: transfusion-dependent thalassemia (TDT), Hb: hemoglobin, TI: Transfusion Independence, SCD: Sickle cell disease, VOC: veno-occlusive crisis, NGS: Next-Generation Sequencing, CHIP: Clonal Hematopoiesis of Indeterminate Potential, Beti-cel: betibeglogene autotemcel, Exa-cel: exagamglogene autotemcel, VAF: variant allele frequency

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Received 30 Apr 2023; Accepted 31 May 2023

is integrated into CD34+ hematopoietic stem cells using lentiviral vectors.

Long-term efficacy and safety of beti-cel were investigated in two phase 1/2 studies, two phase 3 studies (two-year follow-up), and one long-term study (13-year follow-up) by Walters et al.¹ The researchers also used multivariate analysis to determine predictive factors for successful treatment in phase 3 studies. The study included 63 patients with transfusion-dependent thalassemia (TDT) in a median follow-up period of 41.4 months (9-87.5). Busulfan infusion was performed to induce myeloablation, accompanied by beti-cel infusion. Patients who maintained an average Hb above 9 g/dl without packed red blood cell transfusions for 12 consecutive months were considered transfusion-independent (TI).

The mean time it took for neutrophils and platelets to engraft was 23 (13-39) days and 45 (19-191) days, respectively. In phase 3 studies, the level of Hb derived from gene therapy (HbA^{T87Q}), the level of total Hb, and the number of vector copies in the peripheral blood were more stable and durable than in phase 1/2 studies. It was also found that HbA^{T87Q} levels were stable at 6 months at a level above 9 g/dl without the need for red blood cell transfusions.

Transfusion independence was also durable over a 7-year follow-up period, regardless of age or genotype. Moreover, the proportion of patients achieving TI in phase 3 trials was higher (89.5%) than in phase 1/2 (68.2%) and a reduction in markers of ineffective erythropoiesis and iron overload was observed in this portion of patients. In total, there was no need for chelation therapy in 21 out of 37 patients who had recommenced after 2 years.

As for the safety profile, beti-cel ex vivo therapy was also promising. It was found that 18% of patients reported at least one side effect. The occurrence of reported side effects continued up to two years after the infusion. The most common reported side effects were abdominal pain (8%), lower platelet counts (5%), and veno-occlusive liver disease (11%). There was no report of cancer development, insertional oncogenesis, vector-derived replication-competent lentivirus, or clonal predominance.

It has to be mentioned that FDA has approved the use of betibeglogene autotemcel (beti-cel) in patients with transfusion-dependent β -thalassemia. A conditional marketing authorization has also been granted to beti-cel by the European Commission in 2019.

Apart from gene therapy, progress has been made in the supportive treatment of β -thalassemia patients as well. Luspatercept is a novel recombinant protein that preferentially binds to TGF- β superfamily ligands, such as GDF11, GDF8, and activin B. The significant inhibition of the TGF- β signaling promotes the differentiation and maturation of late-stage erythroid precursors such as

erythroblasts.

The BELIEVE study in patients with Transfusion Dependent β -Thalassemia showed a reduction in transfusion burden by at least 33% in patients in the luspatercept group compared with that in the placebo group (70,5% vs 29,5%) during any 12-week interval.² Thus, luspatercept has been approved for the treatment of adult patients with transfusion-dependent β -thalassemia. Real-world data on novel drugs are always interesting. Herein, Delaporta et al. presented the Greek experience of luspatercept use in patients with thalassemia.³

According to data collected from 9 Greek thalassemia units, 102 transfusion-dependent patients with thalassemia and various genotypes were treated with luspatercept (dose: 1-1.25 mg/kg) for a minimum of three months. During the follow-up period, the number of packed red blood cells needed was significantly lower, and the need for transfusions was stable between the first and second three-month intervals. The study also demonstrated that a boosted dose is required in patients with a less favorable response to treatment from the beginning. No statistically significant changes were observed in Hb levels prior to transfusion. However, about half of the patients reduced their required transfusion volume by at least 33% between 13-24 weeks, with only one requiring regular transfusions thereafter. In addition, uric acid (weeks 1 through 36), LDH (weeks 1 through 24), and platelets (weeks 24 to 36) all showed statistically significant increases. About half of patients reported at least one side effect associated with the drug. These included bone pain (35.2%), fatigue (16.7%), headache (8.8%), and periorbital edema (6.9%).

The decision to discontinue treatment was made in 35% of cases, mostly because of no satisfactory response or tolerability issues. It is interesting to note that in this study, the patients who were able to achieve a decrease in transfusion burden equal to or higher than 33% outnumbered those in the clinical trial "Believe" during weeks 13 through 24 (49% vs 21.4%). Even though the results from the use of luspatercept in TDT patients with thalassemia seem promising regarding safety profile and effectiveness, clinical trials with longer follow-up periods and larger samples are required to reach safe conclusions.

Last but not least, Solomou et al. presented clonal hematopoiesis of Indeterminate Potential (CHIP) and its clinical significance in patients with thalassemia.⁴ Even though the survival rate of patients with TDT has significantly improved over the past few years, several factors contribute to high morbidity and mortality, including iron overload, cancer, thromboembolism, and cardiovascular complications, most of which are attributed to oxidative stress. CHIP is determined by the detection of somatic mutations in peripheral blood with a variant allele frequency (VAF) equal to or more than 2% in malignancy-associated

genes, which are commonly found in elderly patients (over 65 years old). CHIP is connected to different types of hematopoietic malignancies, strokes, and cardiovascular complications. The researchers used Next-Generation Sequencing (NGS- Illumina, San Diego, CA) to investigate 95 TDT patients for CHIP presence. Specifically, fifty-eight genes associated with malignancies of the myeloid line were analyzed using 3 bioinformatic tools (Varsome, ClinVar, and Franklin databases).

Study results indicate that 15,8% of the patients had mutations in genes associated with CHIP at a younger age than the general population. In 15 patients, 22 somatic variants were detected [median VAF: 40% (1,5-52%)] in several genes, including ATM, FLT3, ASXL1, MPL, SF3B1, BRAF, BTK, TP53, JAK2, RUNX1, ETV6, and EZH2. According to the researchers, TDT patients with mutations closely associated with myeloid malignancies comprise a significantly higher percentage than the general population. This observation, however, remains unclear regarding clinical significance. It is necessary to conduct further research to determine whether this group of patients with TDT and CHIP is more likely to develop hematological malignancies as a result of long-term stress on hematopoiesis or other complications (cardiovascular events, arrhythmias, strokes).

Regarding sickle cell disease, Frangoul et al. reported their results of gene editing with the use of CRISPR/ Cas technology in patients with sickle cell disease.⁵ Undoubtedly, patients with sickle cell disease benefit from increased fetal hemoglobin levels. A CRISPR/Cas9- based method was used in the case of Exagamglogene autotemcel (or CTX001) to target BCL11A's erythroid enhancer region on autologous CD34+ hematopoietic stem cells. Activating HbF production is the intended outcome. As reported in the pivotal CLIMB SCD-121 trial, a single administration of exa-cel increased HbF and total Hb levels and reduced vaso-occlusive crisis (VOC) frequency.

A total of 31 people (age 12-35 years old) with SCD and ≥ 2 VOCs during the past two years were enrolled in the study. Busulfan was used to conduct myeloablation on patients prior to exa-cel infusion. In the pivotal study, no VOCs were reported by any patient during follow-up (2-32 months after infusion). By the third month after the infusion, mean HbF levels were over 20% with total Hb levels over 11 g/dl, with 11/31 patients experiencing no VOCs after a 12-month period. It is reported that exa-cel infusion did not cause serious side effects. One year after CRISPR/Cas9 editing, the proportion of modified BCL11A alleles has remained stable, indicating hematopoietic stem cells have been successfully modified over the long term.

Today, bone marrow transplantation is the only approved cure for sickle cell disease. Still, it comes with various side effects, such as graft versus host disease, immunosup-

pression, chemotherapy-related side effects, increased infection risk, veno-occlusive liver disease, and gonadal dysfunction. The FDA has been requested to approve CRISPR-based exagamglogene autotemcel for the treatment of sickle cell disease and β - thalassemia. The approval would make it the first human application of CRISPR technology, although being investigated in various other diseases, such as hemophilia, Duchenne muscular dystrophy, α 1-antitrypsin deficiency, malignancy, and hearing impairment. Moreover, since the CRISPR-Cas9 system is derived from bacteria and functions as a defensive mechanism against invasive agents, its therapeutic application in bacterial and viral infections is under investigation, especially in the case of various non-treatable viral infections (HPV, HBV, Epstein-Barr).⁶

Complement activation in sickle cell disease has gained more attention in recent years. This is due to the release of hemoglobin during hemolysis, which activates the complement alternative pathway. In the aftermath, complement activation precipitates a veno-occlusive crisis (VOC) and anemia deterioration. Recently, many authors, including our team,⁷ examined the role of complement in sickle cell disease and found that it may be implicated in hemolysis and sickle cell disease crisis. Thus, Dai et al. presented the study design, which will run and evaluate the role of complement activation and its inhibition in patients with sickle cell disease.⁸

The study is premised on the idea that inhibition of the complement activation cascade should improve anemia and minimize the possibility of VOC. ALXN1820 is a novel humanized bispecific antibody that captures both human albumin and properdin, representing an innovative therapeutic challenge. Properdin is a protein that binds to bacterial and human cell walls, activating the C3 and C5 convertases to generate an attack complex that ruptures the cell membrane. ALXN1820 inhibits the complement activation cascade. Prior testing of an antibody targeting properdin in mice demonstrated that it positively impacted hemolysis and VOCs. A phase 2a open-label trial (NCT04631562) is underway to determine the safety profile and effectiveness of ALXN1820 in patients with sickle cell disease. The antibody was administered subcutaneously, while co-treatment with hydroxyurea did not constitute an inclusion criterion. Voxelotor or crinzalimumab use was not allowed within two months of recruitment. The treatment efficacy will be assessed using complement biomarkers, hemoglobin levels, hemolysis markers, and hemopexin levels. ALXN1820 will be tested in this trial to see if it improves hemolysis and decreases VOCs, as did anti-properdin antibody testing in mice.

Another example of complement inhibition in sickle cell disease is the use of eculizumab, a C5 inhibitor mostly used in paroxysmal nocturnal hemoglobinuria and atypical uremic hemolytic syndrome. Eculizumab has been used in sickle cell disease patients and in crises, bone

marrow necrosis, or thrombotic microangiopathy after allotransplantation of hemopoietic stem cells. In addition, eculizumab has also been used in patients suffering from SCD crises. An essential mechanism of action is the reduction of thromboinflammation, induced by heme. Atypical viruses and encapsulated bacteria are considered the main culprits. The latest research indicates that eculizumab increases meningococcal infection risk by 1000-2000 times regardless of vaccination status. Consequently, infection risk should always be considered when complement inhibition is thought to be the most suitable option.⁹

Lastly, the study of Njoku et al. was selected from six abstracts that examined barriers to high-quality care for sickle cell disease in adults and adolescents.¹⁰ The Sickle Cell Disease Implementation Consortium collected data regarding 2444 patients with sickle cell disease from 8 different areas of the United States. The primary aim of this study was to identify causes of mortality and detect the presence of any clinical phenotype associated with lower survival rates. There were 84 deaths between 2017 and 2022, with 6 of them being trauma-related.

There are multiple causes of increased mortality, including avascular joint necrosis, chronic kidney disease, pulmonary arterial hypertension, sarcoidosis, iron overload, chronic pain, psychological disorders, and hospitalizations for emergency medical conditions (>10 during the last year). In terms of mortality rates, no statistically significant difference was found between those who received hydroxyurea and those who did not. One of the most significant conclusions of the observational study was that sickle cell disease patients have a shorter life span than the general population. In order to minimize complications and all of these factors that place patients with sickle cell disease at risk, an organized and strict surveillance program is essential.

Acknowledgments: None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

Conflict of Interest: The authors have no conflicts of interest to declare.

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