

## Therapeutic approaches in patients with $\beta$ -thalassemia

Mohammad Eini MSc<sup>1</sup>, Mohammad Shoaie MSc<sup>2</sup>, Ebrahim Miri-moghaddam PhD<sup>3,\*</sup>

1- Department of Hematology, faculty of paramedical, Birjand University of Medical Science, Birjand, Iran

2- Department of Medical Biotechnology, Faculty of Medicine, Birjand University of Medical Sciences, Birjand, Iran

3- Cardiovascular Diseases Research Center, Department of molecular medicine, Birjand University of Medical Sciences, Birjand, Iran

\*Corresponding author: Dr Ebrahim Miri-Moghaddam, Cardiovascular Research Centre, Razi Hospital, Faculty of Medicine, Birjand University of Medical Sciences, Birjand, Iran, 9717853577. E-mail: moghaddam4@yahoo.com. ORCID ID: 0000-0001-9435-2450

Received: 04 June 2021

Accepted: 07 September 2021

### Abstract

Beta-thalassemia ( $\beta$ -thal) is a congenital hemoglobinopathy explained by a decreased level ( $\beta^+$ ) or absence ( $\beta^0$ ) of  $\beta$ -globin gene expression. Microcytic hypochromic anemia and various clinical symptoms comprising severe anemia to clinically nonsymptomatic features. Treatment with an ordered blood transfusion and iron chelator agents can decrease transfusion iron overload that causes normal maturation. These patients also are at high risk for secondary iron overload because of erythropoietin (GF15-TW5G1) release from erythroblasts resulting in erythroid hyperplasia. Based on the previous studies, chemicals such as hydroxyurea and 5-azacytidine are useful in treating  $\beta$ -hemoglobinopathy, including  $\beta$ -thal and sickle cell disease (SCD). Regarding both side effects and lifelong treatment of these chemical components, researchers have recently regarded gene-based treatments. These techniques, such as micro RNA gene silencing, viral-mediated gene editing, and clustered regulatory interspaced short palindromic repeats (CRISPR)-CAS9 systems, are the most commonly used gene therapy methods. Nowadays,  $\gamma$ -globin (fetal globin) gene reactivation is one of the most popular treatments for  $\beta$ -thal. Researches showed that these gene modification methods for  $\gamma$ -globin gene reactivation are also useful in increasing hemoglobin F (HbF) and helping patients with  $\beta$ -thal. In this review study, new therapeutic approaches to manage this disorder are regarded.

**Keywords:** Beta-thalassemia, Genetic therapy, Hemoglobinopathy

### Introduction

Beta-thalassemia ( $\beta$ -thal) is a congenital monogenic disorder caused by a decrease or absence of the  $\beta$ -globin chain synthesis (1). The suspected mutation carriers rate is estimated to be 1–5 %, worldwide (2). According to the WHO report, about 50000-100000 new patients annually join the major thalassemia society (3). Several mutations in the  $\beta$ -globin gene or regulatory elements are identified that cause  $\beta$ -globin decreased production or silencing. Finally, an imbalance in globin chains is responsible for ineffective erythropoiesis and chronic hemolytic anemia (1). Prenatal diagnosis (PND) is a correct part of a social control program for thalassemia. Unawareness of being minor (due to the lack of the screening program before marriage) and unawareness of PND

importance and process was reported as the most causes of new affected births in South-East of Iran (4). Premarital screening tests are considered a necessary section of healthcare service provision in areas with a high frequency of hemoglobin disorders (5). However, it may be required for the couples, which were not screened before marriage to undergo Pre-pregnancy (preconception) screening. Identification and detection of these mutations can play an essential role in disease control by prenatal diagnosis. Some countries, such as Greece, Italy, and Cyprus have decreased the rate of thalassemic newborns to near zero (6). Due to cultural and ethical reasons, the fetuses' curettage (abortion) with a  $\beta$ -thal major is not legal and ethical in some areas of the world. Therefore, prenatal diagnosis cannot reduce the birth

of newborns with thalassemia and other methods such as preimplantation genetic diagnosis must be used (7, 8). During the use of the PND technique, it should be noted that the existence of maternal cell contamination in fetus specimens demonstrates a risk for prenatal misdiagnosis when molecular techniques are used to detect genetic disorders. When the typical haplotype from motherhood cells is mistaken as fetus cells, a fetus with homozygous thalassemia may be misidentified as heterozygous thalassemia (9, 10). Blood transfusion is a traditional treatment for  $\beta$ -thal that improves anemia and lifespan (11). One unit packed cell transfused to the recipient involves 200 mg iron and causes iron overload because the iron regulatory system cannot excrete an excessive amount of iron from the bloodstream (12). This high level of iron precipitates in the parenchymal tissue such as the heart, gonads, endocrine glands, and liver that causing organ failure and growth retardation (13). The transfused blood components antigens may also activate the recipients' immune system and cause alloantibodies production. In the next transfusions, these produced antibodies bind to specific antigens and account for the hemolytic transfusion reaction. Blood transfusion and iron chelation, which are used as routine treatments can improve the lifestyle and survival of the  $\beta$ -thal patients. Deferoxamine, Deferasirox, and Deferiprone are prescribed as iron chelators in transfusion-dependent patients and alleviate iron overload (14). Desferrithiocin, Deferitrin, and Amlodipine are new iron chelators in clinical trials (15). Therefore, researchers are looking for other approaches due to the high costs imposed on the health care systems and patients and the side effects of blood transfusion. Today, Hematopoietic stem cell transplantation (HSCT) is proposed as a definitive treatment for  $\beta$ -thal patients. Although the HSCT is a curative method for the patients resulting in improved

quality of life and long-term treatment for these patients, it has limitations such as limited availability of HSC-matched donors, limits on the Human Leukocyte Antigen (HLA) system compatibility, and Graft Vs Host Disease (GVHD). As HLA-matched donor is not available for many patients, induced pluripotent stem cells (iPSC) are a useful treatment for these cases. Takahashi et al. demonstrated that OCT3/4 (organic cation transporter 3), KLF4 (Krupple Like Factor 4), SOX2 (SRY-Box Transcription Factor 2), and MYC (V-Myc Avian Myelocytomatosis Viral Oncogene Homolog) transcription factors could reprogram the somatic cells to their pluripotent state and clinicians can later use them for gene manipulation instead of Hematopoietic Stem Cell (HSC) (16). By using immune-suppressive drugs, clinicians have shown that these agents can control GVHD and other immune reactions. Anyway, taking these medications is very toxic for pediatric class III patients (patients with splenomegaly  $> 2$ cm, irregular chelation history, and portal Fibrosis) (17). Due to these limitations, HSCs transplantation is not a widely used curative method (2). Due to the complications of blood transfusions, iron chelation, and bone marrow transplantation, induction of fetal hemoglobin (HbF) in adult Hematopoietic progenitor cells (HPCs) has the potential for the best clinical advantage in patients with harmful point mutations in the  $\beta$ -globin cluster such as  $\beta$ -thal and sickle cell disease (SCD).

Chemical substances that can induce HbF synthesis in adult erythroid progenitors and some transcription factors have been recognized. Gene therapy and gene editing are methods to attain stable introduction of a correct  $\beta$ -globin gene into the patient's HSCs that can synthesis a normal  $\beta$ -globin chain (18). They can correct ineffective erythropoiesis, reduce hemolysis, and obviate the need for regular blood transfusion (19). This study presents new prevention methods and treatment of  $\beta$ -thal

and its complications to provide a review for researchers to further studies on this topic.

## Management

### 1. Fetal hemoglobin induction

Erythroid progenitor's express different hemoglobin types at various developmental steps during erythroid maturation, including embryonic, fetal, and adult, respectively. The overall tetramer structures consist of 2  $\alpha$ -Like and two  $\beta$ -like globin chains.  $\beta$ -globin switching completes a little while after birth when  $\gamma$ -globin gene expression is effectively silenced, and  $\beta$ -globin (HBB) becomes the most highly expressed locus. Epigenetic mechanisms usually regulate hemoglobin switching consisting of modifications to higher-order chromosome structure, DNA methylation, and histone modifications (20). By using chemical treatments or genetic manipulations such as inhibition of methylation and acetylation in DNA that affects histone methyltransferase (EHMT2 and EHMT1), histone arginine methyltransferase (PRMT5), methyl-cytosine binding protein (MBD2), and histone deacetylases (HDACs) have increased  $\gamma$ -globin synthesis (21). HDAC enzymes remove acetyl groups from  $\epsilon$ -N-acetyl-lysine amino acid residues in the histones. It has been reported that inhibition of HDACs by chemical compounds results in hyperacetylation of histones H3 and H4 that causes an open chromatin configuration, binding of transcription factors, and consequent induction of gene transcription (22).

The conception of the molecular foundation for  $\gamma$ -globin gene silencing in the adult stage has been recognized by some  $\gamma$ -globin gene expression inhibitors, which affect the promoter or the interactions that interrupt attachment of the locus control region to the gene promoter. The transcription factors BCL11A (BAF Chromatin Remodeling Complex Subunit BCL11A), GATA1 (Globin Transcription

Factor 1), FOG1 (Zinc Finger Protein, FOG Family Member 1), SOX6 (SRY-Box Transcription Factor 6), LSD1 (Lysine-specific histone demethylase 1A), and KLF1, are essential in the  $\gamma$ -globin switching process. It is well known that the clinical severity of  $\beta$ -thal is decreased in people whose fetal globin synthesis significantly raised, commonly from 20–30% (23-26).

#### 1.1 Chemical fetal globin induction

In order to enable a substance that induces  $\gamma$ -globin synthesis to function until the apoptosis pathway is irreversibly developed, the best hematologic rectification of  $\beta$ -thal requires improvement in proliferation or survival in erythroid cells. Chemotherapeutic factors that induce HbF synthesis, such as decitabine, 5-azacytidine, and Hydroxyurea (HU) inhibit cell growth and cause cell proliferation stoppage which is known to induce irrevocable programmed cell death in tumor cells (27). Several short-chain fatty acids (SCFAs) specifically induce  $\gamma$ -globin gene expression and in some people can elevate the yield of  $\gamma$ -globin synthesis. The effect of SCFAs in  $\gamma$ -globin production does not need HDAC inhibitor activity, but HDAC inhibitor is usually a potent fetal globin inducer. However, those several SCFAs that inhibit HDACs usually inhibit cell proliferation. Unlike chemotherapeutic agents, the SCFAs are not mutagenic. Previous studies demonstrated that Arginine Butyrate (AB) and SCFA induced  $\gamma$ -globin expression by reducing HDAC3 level, which increased fetal globin to 30% and 60%, respectively. SCFAs induce  $\gamma$ -globin gene expression by displacement of an HDAC3-NCoR repressor complex. Butyrate by another mechanism can increase fetal globin through the activation of P38/MAPK (*p38* mitogen-activated protein kinases) that involves GATA1 and NF-E2 (Nuclear Factor, Erythroid 2) transcription factors. Hydroxyurea (HU) is a cytotoxic agent ribonucleotide reductase inhibitor and

antimetabolite with the most extraordinary fetal globin induction (28). HU can increase fetal globin in  $\beta$ -thal and SCD patients. The main limitation of HU usage for  $\beta$ -thal is that greater demand for fetal globin production to achieve a balanced globin chain may require a dosage increase that causes cytopenia (29). Although the mechanism of HU-related fetal globin induction is still unclear, the most accepted hypothesis is that HU accounts for a "stress erythropoiesis" response that causes an increase of HbF because erythroblasts would not have sufficient time for development and silencing of the  $\gamma$ -globin gene.

HU increases  $\gamma$ -globin expression by changing the methylation and demethylation patterns. Changing in the methylation does not increase  $\gamma$ -globin significantly, albeit a decrease in methylation of CpG islands in the  $\gamma$ -globin gene promoter is associated with fetal globin reactivation (30) (Figure 1).

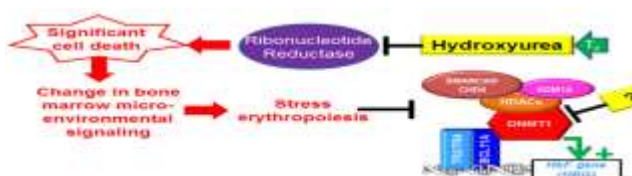


Figure 1- Induction of fetal globin with HU by chromatin remodeling in the recovery phase that causes HbF induction (82).

Another suggested mechanism for fetal globin induction by HU is the effects of the Nitric Oxide (NO) and cGMP (cyclic guanosine monophosphate) pathway. In this mechanism, HU stimulates NO production that provokes increasing of intracellular cGMP which later results in HbF induction by PKG (cGMP-dependent protein kinase). HU can increase  $\gamma$ -globin mRNA expression about 2-9-folds and also the  $\alpha/\beta$  ratio. According to some studies, the response rate to HU ranged from 60 to 100% (21). The DNA methyltransferase (DNMT) inhibitors such as Azacytidine (5-Aza) and 2'-deoxy-5-azacytidine (Decitabine) are cytidine agonists that enter the DNA

strands and inactivate the DNMT enzymes by proteasome degradation. DNMT inhibitor agents cause methylation of fetal globin gene expression suppressor transcription factors or fetal globin regulatory elements and lead to fetal globin reactivation (31). Butyrate is an HDAC inhibitor that increases fetal globin level by epigenetic mechanisms followed by reactivation of silent genes in fetal globin regulatory networks (32, 33). Lenalidomide and Pomalidomide are immunomodulatory and antiangiogenic factors used in refractory and relapsed Multiple Myeloma. The studies confirmed that Pomalidomide could reactive a fetal-like erythroid production by influencing the regulators of  $\gamma$ - and  $\beta$ -globin transcription such as KLF1, SOX6, BCL11A, IKZF1 (IKAROS Family Zinc Finger 1), and LSD1 (34, 35). Tranylcypromine (TCP) is a monoamine oxidase inhibitor reported as a  $\gamma$ -globin inducer recently (36). TCP can increase total Hb from 4.6% to 31% in vitro (37). Forkhead-box-O3 (FoxO3) is a critical TF that upregulates and increases antioxidant enzyme expression and protects cells from oxidative stress in the early stage of erythropoiesis (38, 39). FoxO3 gets phosphorylated and inactivated by EPOR-PI3K/AKT/mTOR (Erythropoietin receptor-phosphatidylinositol-3 kinase/protein kinase B/ mammalian target of rapamycin) and is translocated out of the nucleus. In  $\beta$ -thal intermedia mice, the mTOR complex is forcibly activated, FoxO3 is downregulated and these lead to oxidative damage and intramedullary hemolysis (40). Rapamycin is an mTOR inhibitor that significantly increases cell maturation and  $\beta$ -globin production in erythroid cells. In erythroblast cultured cells from  $\beta$ -thal patients, rapamycin increases  $\gamma$  globin expression (41). Metformin which is approved for diabetes type 2, could act as a FoxO3 and  $\gamma$ -globin inducer (42). Growth Differentiation Factor 11 (GDF11) is a member of the TGF $\beta$  (Transforming growth factor B)

superfamily that is increased in  $\beta$ -thal patients and blocks the terminal erythroid maturation stage by amplification of  $\alpha$  chain precipitation and ROS (Reactive oxygen species) damage. Luspatercept is a fusion protein driven from the extracellular domain of human activin receptor type II and Fc fragment of IgG1 (Insulin Like Growth factor) that works in the late maturation stage of erythroid progenitor and improves ineffective erythropoiesis. In other words, this monoclonal antibody, recently approved by FDA, binds to GDF11 (Growth Differentiation Factor 11) and inhibits the SMAD (Transforming Growth Factor-Beta Signaling Protein) signaling pathway (43, 44). Luspatercept can decrease transfusion requirement and liver iron concentration in transfusion dependence  $\beta$ -thal patients and improves life quality in non-transfusion dependent patients (15). Transferrin is a positive acute-phase protein synthesized by the liver and has an essential role in plasma iron transport. Studies have been shown that a decrease in transferrin saturation can be beneficial for  $\beta$ -thal patients, so the administration of appo-transferrin (transferrin without Fe<sup>3+</sup>) can decrease serum iron, increase Hb production, and normalize RBC destruction (45). Fagonia Cretica (FC) is a member of the Zygophyllaceae family used as herbal medicine. FC is a P53 (Tumor protein P53) and FOXO3 inducer used for Myelodysplastic Syndrome (MDS) and Congenital Erythropoietic Porphyria (CEP) patients who did not respond to treatment (46). Rashid A. Seyal et al. (47) showed that the prescription of FC for  $\beta$ -thal patients who did not tolerate blood transfusion can significantly improve HbA and HbA<sub>2</sub> and decrease HbF level after about ten months of treatment.

### 1.2 Gene manipulation

Although HSCT is the only curative method for thalassemia treatment, it has a significant risk of morbidity and mortality in the HSCT recipient (48). Gene manipulation with the goal of  $\beta$ -thal

treatment developed a lot of investigations. Gene manipulation for  $\beta$ -thal treatment is divided into gene therapy and gene editing (49-51).

### Gene therapy

Gene therapy had been done in the 1980s for the first time for hemoglobinopathy. This strategy aims to deliver normal globin genes into stem cells by suitable vectors. Investigators have been trying to overcome the main complexities in this field; including the selection and design of efficient, harmless, and safe vectors, the safe and efficient HSC mobilization from bone marrow to the blood circulation; by G-CSF (granulocyte-colony stimulating factor) or GM-CSF (granulocyte monocyte-stimulating factor), genetic manipulation, transplant manipulated-HST into the patient, and combination and assembly of different regulatory DNA elements associated with high expression of  $\gamma$ -globin, without interference with the endogenous gene around the transgene integration site (51). The gene carriers are usually viruses. Among them, lentiviral vectors are the best choice for transfection (exogenous gene transition to mammalian cells) because retroviral and adenoviral vectors are unsafe for HST and may cause infection. Adeno-associated viruses have tiny genomes and cannot carry the complete regulatory DNA elements necessary for a high-level of  $\gamma$ -globin expression.

On the other hand, lentiviral vectors allow the insertion of complex DNA elements, including LCR-HS (locus control region),  $\beta$ -globin promoter, 3' enhancer, and separate elements (insulators) that cover the exogenous gene from repressive chromatin. The genes near the integration area should be protected from being activated by the transferred gene regulatory elements. If the genes near the insertion site are affected by the regulatory element of transferred DNA or this insertion activates the proto-oncogenes and

stimulation of proliferation, it can result in leukemia and MDS (52, 53).

The critical thing in vector design is that the regulatory elements such as a promoter, enhancer, etc., should be specified for erythroid progenitors. Researchers demonstrated that LCR-HS2 and LCR-HS3 could induce a high level of expression in vitro in transgenic mice.

Zynteglo is a newly approved gene therapy method based on Lentiviral vector. It consists of autologous CD34+ cells encoding the  $\beta$ A-T87Q-globin gene. It is a one-time gene therapy appropriate for 12 years old or older transfusion-dependent  $\beta$ -thal patients who do not have a  $\beta$ o/ $\beta$ o genotype and do not have an HLA matched related HSC donor (54) (Figure2).

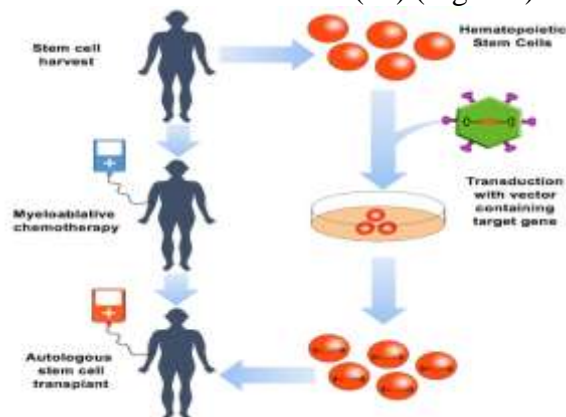


Figure 2- Human HSC gene therapy (51).

### 1.2.1 Gene editing

Gene editing is another thalassemia treatment method based on targeting a specific nucleotide sequence in the human genome and repairing double-strand breaks by Non-Homologous End Joining (NHEJ) and homology-directed repair (HDR). The NHEJ repairs system and changes the genomes by deletion or insertion. It removes a regulatory element or protein expression inhibition. Still, HDR is based on double-strand or single-strand break with nuclease in mutant sequence and replaces the Wild-Type sequence transferred to the cell by a viral vector (51, 55, 56). The nuclease binds to the specific sequence in the genome by combining with either synthetic DNA binding protein

such as Zinc Finger (ZF), Transcription Activator-Like Effector (TALE), or gRNA that are existed in the clustered regulatory interspaced short palindromic repeats (CRISPR)-CAS9 system (57) (Figure 3).

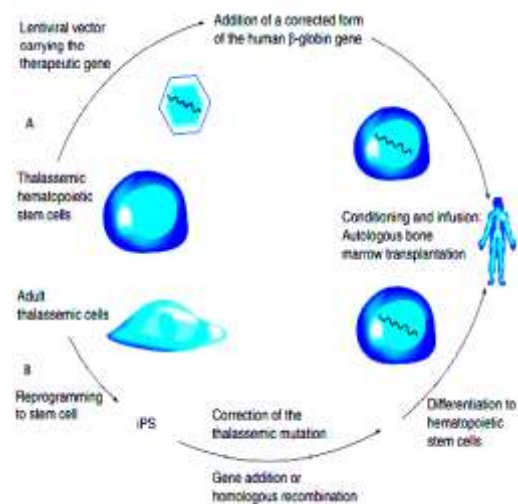


Figure 3. Gene therapy and cell therapy. (a) The normal gene is transferred to the HSC by a Lentiviral vector. (b) Reprogramming somatic cell (IPS) to stem cells (83)

ZF and TALE usually fuse with Fok1 (a nuclease) and produce ZF nuclease and TALEN nuclease (57). TALEN binds to about 15 bp<sub>s</sub> in DNA, and gRNA in the CRISPR-CAS9 system can recognize 18 bp<sub>s</sub> and binds to a specific sequence in the genome (51, 58). The identified sequence length is directly related to the specific binding in the genome and causes specific DNA breakdown. Due to the probability of chromosomal rearrangement, these methods have some limitations, but among them, CRISPR-CAS9 has more advantages because of the straightforward design and cost-effectiveness. One type of CRISPR-CAS9 system that contains a fusion protein of CAS9 nuclease-transaminase is used for site-specific base editing in the genome. Researchers can replace the mutant base with the correct base at a specific site (59). Hereditary persistence of fetal hemoglobin (HPFH) is a congenital disorder with high HbF expression compared with a normal individual of the same age and sex. A 13 kb deletion in the

5' region of the  $\gamma$ -globin gene juxtaposes to  $\gamma$ -globin promoter close to the 3' enhancer of  $\beta$ -globin resulted in the deletion of negative trans-acting element located in the upstream of the  $\gamma$ -globin gene. Ye et al. (60) have shown that erythroblast cells with 13 kb deletion overexpress the  $\gamma$ -globin than normal cells. Direct repeat erythroid definitive (DRED) complex is a transcription repressor protein that binds to 13 bp<sub>s</sub> in DNA located at 100 bp<sub>s</sub> upstream of the  $\gamma$ -globin gene, by TR2, TR4, and BCL11A. HDAC and LSD1 are two co-repressors for the DRED complex. In other words, DRED binds to the CCAAT sequence by BCL11, TR2, and TR4, and makes HDAC and LSD1 recruitment possible. In the next step, HDAC and LSD1 cause  $\gamma$ -globin silencing (61). The study done by Li et al. demonstrated that a similar deletion sequence in mice by CRISPR-CAS9 leads to elevation of  $\gamma$ -globin expression (62). BCL11A is a transcription factor that is important for several cellular functions, and it is an essential repressor for  $\gamma$ -globin gene expression. BCL11A binds to the  $\gamma$ -globin promoter and causes  $\gamma$ -globin switching in adult erythroblasts (63-65). In other words, the BCL11A is a central factor for  $\gamma$ -globin switching that co-express and directly interact with the  $\beta$ -globin loci with SOX6 in association with deacetylase complex and Mi-2/nucleosome that account for  $\gamma$ -globin silencing (66). Targeting this TF is a method for  $\gamma$ -globin induction, but it is crucial that targeting be restricted to the erythroid cell line. Several cis-acting elements locate in BCL11A loci, such as GATA as a motif associated with erythroid BCL11A enhancer. Disruption of the coding region of BCL11A, in contrast to increasing  $\gamma$ -globin expression, leads to reduced cell proliferation of erythroid progenitor (67). Targeting the GATA motif is responsible for decreased BCL11A, elevated HbF without effect on cell proliferation (68). Viral Protein 64 (VP64) is a minimal activation domain of HSV-16 that is a tetrameric protein. Fused

VP64 with ZF is specific for the  $\gamma$ -globin gene promoter done by Wilber. Lentiviral transferring of VP64 fused with ZF causes elevation of HbF expression by about 20% (69). LCR is located upstream of the  $\beta$ -Like gene cluster and enhances  $\beta$ -like gene expression by looping mechanism. Ldb1 is a factor that mediates the interaction between LCR and globin genes during the looping mechanism, which required GATA1/Tal1. Fused Ldb1 with specific ZF for  $\gamma$ -globin genes enhances loop formation artificially and increases  $\gamma$ -globin expression (70,71) (Figure 4).

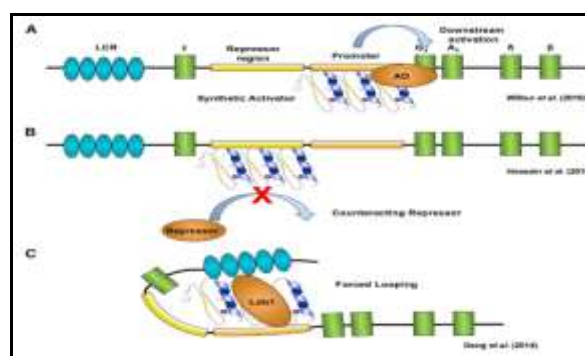


Fig. 4. Using ZF for  $\gamma$ -globin induction. (A) Synthetic ZF domain that is specific for  $\gamma$  globin is fused with activator domain such as VP64. This fusion protein binds to the gamma gene promoter specifically and induces  $\gamma$ -globin expression. (B) Specific ZF can bind to the known repressor site in the  $\gamma$ -globin gene and accounts for the increased HbF. (C) A specific ZF domain fuses with the dimerization domain of Ldb1. Ldb1-ZF complex facilitates chromatin looping, LCR near the  $\gamma$ -globin promoter that causes HbF expression (51).

Micro RNA (miRs) usually have an essential role in  $\gamma$ -globin gene switching through interaction by  $\gamma$ -globin regulatory TFs and changing the expression during development. For example, LIN28B (Lin-28 Homolog B) is a protein that targets the let-7 family and has an inhibitory effect on BCL11A expression by downregulated expression of this gene. Overexpression of LIN28B can decrease BCL11A and elevate  $\gamma$ -globin expression (72). The miR-486-1-3p also causes  $\gamma$ -globin induction by decreasing BCL11A expression (73). Today, researchers are trying to increase HbF by destroying inhibitory or silencer

factors that inhibit  $\gamma$ -globin expressions such as BCL11A, KIF1, SOX6, and MYB by gene-editing methods.

## 2. Alternative method

### 2.1 Janus Kinase 2 inhibitor

Erythropoietin (EPO) is an important growth factor involved in erythropoiesis. Most of it is secreted by the kidney and rarely by the liver in adults (74). Janus Kinase 2 (JAK2) is a kinase that has an essential role in EPOR signaling by mediated conserved cytoplasmic EPOR tyrosine residue phosphorylation restricted by EPO signaling in erythroblasts. EPO binds to EPOR in erythroblasts and induces JAK2-STAT5 (signal transducer and activator of transcription factor 5) cell signaling. Jak2 is activated by auto-phosphorylation and induces STAT5 to get dimerized and mainly causes increased BCL2 expression (75). In the thalassemic mice, using JAK2 inhibitors like Ruxolitinib that prescribed for polycythemia vera and myelofibrosis balances proliferation and differentiation and improves ineffective erythropoiesis (76).

### 2.2 Increase hepcidin expression

In normal conditions, erythropoiesis needs hematinics such as Iron (Fe), B12, and folic acid (B9). Fe is an essential key element in hemoglobin production. Iron intake is controlled by two mechanisms, including controlling the translation level

by iron regulatory protein-iron regulatory element (IRP-IRE) and gene expression level (hepcidin: a positive acute-phase protein produced predominantly by the liver). A low level of hepcidin expression in the brain, macrophage, adipocyte, and other organs, may be necessary for local iron regulation. This hormone has 22 amino acids, inhibits iron release to plasma by ferroportin internalization and induces its degradation by the proteasome in the cytoplasm. Hepcidin gene expression is controlled by plasma transferrin level, erythropoerons (TWSG1 and GDF15 that are secreted by erythroblasts), an inflammatory cytokine (IL6), and matriptase 2 in hepatocytes (77). Thalassemia intermedia is characterized by erythroid hyperplasia (increased erythropoerons) and ineffective erythropoiesis (increased iron turnover, alpha chain precipitation, and ROS production) (Figure 5). Induced Hepcidin expression accounts for decreased iron absorption, ameliorated IE, increased Hb, and improved splenomegaly (78). Mini-hepcidin (a short peptide that can mimic hepcidin function and decrease iron absorption), matriptase2 inhibitor, the elevation of plasma transferrin, and erythropoerons inhibitor could be used to reduce iron intake by hepcidin induction (79-81) (Figure 6).

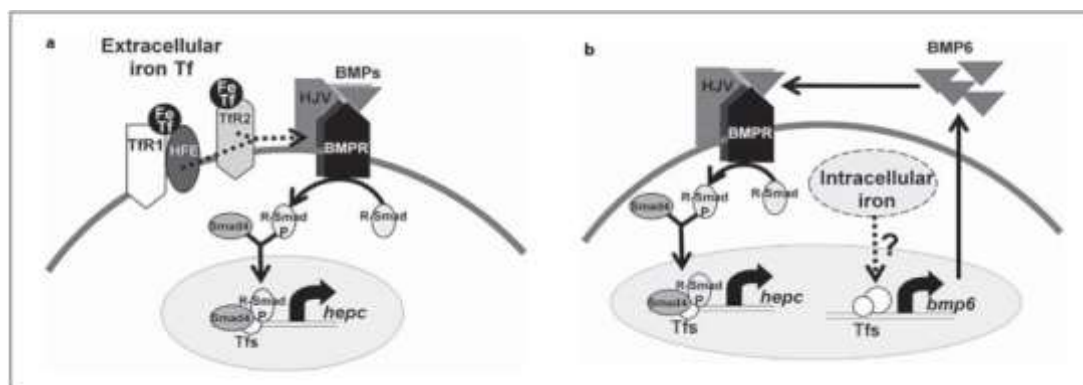


Fig. 5. A schematic model of hepcidin gene expression control in hepatocyte cells by Iron-transferrin (a) and intracellular iron (b). (a) When the holo-transferrin (iron-Tf) is increased in blood circulation, it binds to Tfr1 in the hepatocyte membrane, and HFE disassociates from Tfr1 and binds to Tfr2. Tfr2-HFE complex interacts with HJV-BMP, and Smad signaling gets activated that account for hepcidin expression. (b) Accumulation of intracellular iron accounts for increased BMP6 expression that binds to HJV-BMP and causes increased hepcidin Expression (84).



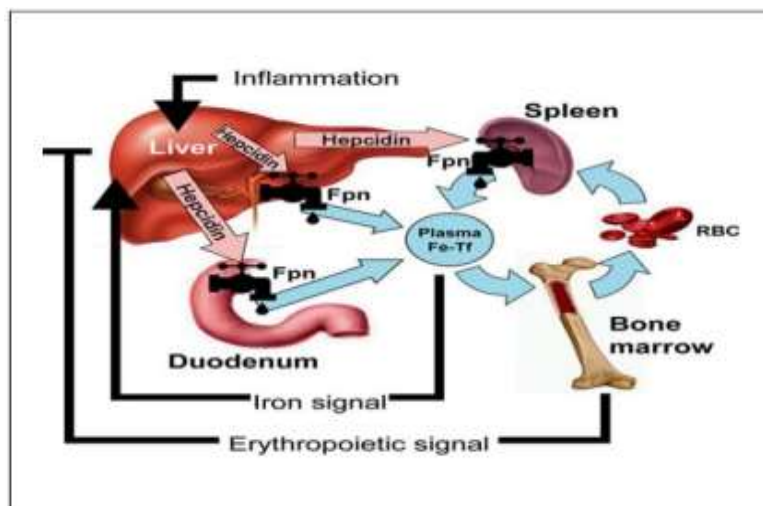


Fig. 6. The role of hepcidin in Fpn expression in enterocytes and Iron level in the bloodstream (84).

## Conclusion

Treatment of  $\beta$ -thalassemia as a prevalent monogenic disorder that needs life-long treatment remains challenging, and many treatment methods have been performed to improve patients' life quality. Screening couples before marriage and PND may be the best approaches to prevent newly affected births and to reduce the high frequency around the world. HCST and blood transfusion are suggested treatment methods for  $\beta$ -thal patients, but these treatments have limitations, as discussed. Nowadays, many studies are focused on gene therapy and gene editing for treating genetic disorders, especially thalassemia and znteglo, an approved treatment, is now available. Induction of fetal globin is a new approach to overcome  $\beta$ -thal using gene therapy methods and chemical substances. These advances have improved the patient's quality of life, and hope to discover more effective treatment methods.

## Conflict of interest

There is no conflict of interest

## References

- Galanello R, Origa R. Beta-thalassemia. *Orphanet J Rare Dis* 2010; 5(1): 1-5.
- El-Beshlawy A, El-Ghamrawy M. Recent trends in treatment of thalassemia. *Blood Cells Mol Dis* 2019; 76: 53-58.
- Modell B, Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. *Bull World Health Organ* 2008; 86: 480-487.
- Miri-Moghaddam E, Naderi M, Izadi S, Mashhadi M. Causes of new cases of major thalassemia in Sistan and Balouchistan province in South-East of Iran. *Iran J Public Health* 2012; 41(11): 67-71.
- Kashfi SM, Mohaghegh Sharifi E, Rakhshani T, Hosseini SS. Tests for the Prevention of Thalassemia Major in the Couples Referring to Behbahan Health Center, Iran During 2006-2016. *J Hum Environ Health Promot* 2018; 4(3): 116-120.
- Miri-Moghaddam E, Zadeh-Vakili A, Rouhani Z, Naderi M, Eshghi P, Khazaei Feizabad A. Molecular basis and prenatal diagnosis of  $\beta$ -thalassemia among Balouch population in Iran. *Prenat diagn* 2011; 31(8): 788-791.
- Miri-Moghaddam E, Zadeh-Vakili A. Profile of  $\beta$ -Thalassemia and its Prenatal Diagnosis in Khorasan-E-Jonobi Province, Iran. *Hemoglobin* 2012; 36(5): 456-463.
- Traeger-Synodinos J. Preimplantation genetic diagnosis, an alternative to conventional prenatal

- diagnosis of the hemoglobinopathies. *Int J Lab Hematol* 2013; 35(6): 571-579.
9. Stojilkovic-Mikic T, Mann K, Docherty Z, Mackie Ogilvie C. Maternal cell contamination of prenatal samples assessed by QF-PCR genotyping. *Prenat Diagn* 2005; 25(1): 79-83.
  10. Li D-Z, Yang Y-D. Invasive prenatal diagnosis of fetal thalassemia. *Best Pract Res Clin Obstet Gynaecol* 2017; 39: 41-52.
  11. Borgna-Pignatti C. Modern treatment of thalassaemia intermedia. *Br J Haematol* 2007; 138(3): 291-304.
  12. Taher AT, Saliba AN. Iron overload in thalassemia: different organs at different rates. *Hematology* 2017; 2017(1): 265-271.
  13. Wahidiyat PA, Liauw F, Sekarsari D, Putriasih SA, Berdoukas V, Pennell DJ. Evaluation of cardiac and hepatic iron overload in thalassemia major patients with T2\* magnetic resonance imaging. *Hematology* 2017; 22(8): 501-507.
  14. Kontoghiorghe CN, Kontoghiorghe GJ. Efficacy and safety of iron-chelation therapy with deferoxamine, deferiprone, and deferasirox for the treatment of iron-loaded patients with non-transfusion-dependent thalassemia syndromes. *Drug Des Devel Ther* 2016; 10: 465-481.
  15. Manglani M.V, Kini P.S. Management of  $\beta$ -thalassemia—Consensus and controversies. *Pediatr Hematol Oncol* 2017; 2(4): 94-97.
  16. Li M, Cascino P, Ummarino S, Di Ruscio AJC. Application of induced pluripotent stem cell technology to the study of hematological diseases. *Cells* 2017; 6(1): 7-20.
  17. Bhukhai K, Leboulch P, Payen E. Current and future alternative therapies for beta-thalassemia major. *Biomed J* 2016; 39(1): 24-38.
  18. Gaj T, Gersbach CA, Barbas III CF. ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. *Trends biotechnol* 2013; 31(7): 397-405.
  19. Karponi G, Zogas N. Gene therapy for Beta-Thalassemia: updated perspectives. *Appl Clin Genet* 2019; 12: 167-180.
  20. Wilber A, Nienhuis AW, Persons DA. Transcriptional regulation of fetal to adult hemoglobin switching: new therapeutic opportunities. *Blood* 2011; 117(15): 3945-3953.
  21. Paikari A, Sheehan VA. Fetal haemoglobin induction in sickle cell disease. *Br J Haematol* 2018; 180(2): 189-200.
  22. Pace BS, Zein S. Understanding mechanisms of  $\gamma$ -globin gene regulation to develop strategies for pharmacological fetal hemoglobin induction. *Dev Dyn* 2006; 235(7): 1727-1737.
  23. Bauer DE, Kamran SC, Lessard S, Xu J, Fujiwara Y, Lin C, et al. An erythroid enhancer of BCL11A subject to genetic variation determines fetal hemoglobin level. *Science* 2013; 342(6155): 253-257.
  24. Norton LJ, Funnell AP, Burdach J, Wienert B, Kurita R, Nakamura Y, et al. KLF1 directly activates expression of the novel fetal globin repressor ZBTB7A/LRF in erythroid cells. *Blood adv* 2017; 1(11): 685-692.
  25. Yi Z, Cohen-Barak O, Hagiwara N, Kingsley PD, Fuchs DA, Erickson DT, et al. Sox6 directly silences epsilon globin expression in definitive erythropoiesis. *PLoS Ggenet* 2006; 2(2): 129-139
  26. Ginder GD. Epigenetic regulation of fetal globin gene expression in adult erythroid cells. *Transl Res* 2015; 165(1): 115-125.
  27. Lohani N, Bhargava N, Munshi A, Ramalingam S. Pharmacological and molecular approaches for the treatment of  $\beta$ -hemoglobin disorders. *J Cell Physiol* 2018; 233(6): 4563-4577.
  28. Iqbal A, Ansari SH, Parveen S, Khan IA, Siddiqui AJ, Musharraf SG. Hydroxyurea treated  $\beta$ -thalassemia children demonstrate a shift in metabolism towards healthy pattern. *Sci Rep* 2018; 8(1): 1-9.

29. Sankaran VG. Targeted therapeutic strategies for fetal hemoglobin induction. *Hematology* 2010, 2011; 2011(1): 459-465.
30. Pule GD, Mowla S, Novitzky N, Wiysonge CS, Wonkam A. A systematic review of known mechanisms of hydroxyurea-induced fetal hemoglobin for treatment of sickle cell disease. *Expert Rev Hematol* 2015; 8(5): 669-679.
31. Gnyszka A, Jasrrebsji z, Flis S. DNA methyltransferase inhibitors and their emerging role in epigenetic therapy of cancer. *Anticancer Res* 2013; 33(8): 2989-2896.
32. Collins AF, Brusilow W. Oral Sodium Phenylbutyrate Therapy in Homozygous  $\beta$  Thalassemia: A Clinical Trial. *Blood* 2011; 85(1): 43-49.
33. Hines P, Dover GJ, Resar LM. Pulsed-dosing with oral sodium phenylbutyrate increases hemoglobin F in a patient with sickle cell anemia. *Pediatr Blood Canc* 2008; 50(2): 357-359.
34. Moutouh-de Parseval LA, Verhelle D, Glezer E, Jensen-Pergakes K, Ferguson GD, Corral LG, et al. Pomalidomide and lenalidomide regulate erythropoiesis and fetal hemoglobin production in human CD34+ cells. *J Clin Invest* 2008; 118(1): 248-258.
35. Dulmovits BM, Appiah-Kubi AO, Papoin J, Hale J, He M, Al-Abed Y, et al. Pomalidomide reverses  $\gamma$ -globin silencing through the transcriptional reprogramming of adult hematopoietic progenitors. *Blood* 2016; 127(11): 1481-1492.
36. Shi L, Cui S, Engel JD, Tanabe O. Lysine-specific demethylase 1 is a therapeutic target for fetal hemoglobin induction. *Nat med* 2013; 19(3): 291-294.
37. Cui S, Engel JD. Reactivation of fetal hemoglobin for treating  $\beta$ -thalassemia and sickle cell disease. *Adv Exp Med Biol* 2017; 1013: 177-202.
38. Liang R, Ghaffari S. Advances in understanding the mechanisms of erythropoiesis in homeostasis and disease. *Br J Haematol* 2016; 174(5): 661-673.
39. Marinkovic D, Zhang X, Yalcin S, Luciano JP, Brugnara C, Huber T, et al. Foxo3 is required for the regulation of oxidative stress in erythropoiesis. *J Clin Invest* 2007; 117(8): 2133-2144.
40. Zhang X, Campreciós G, Rimmelé P, Liang R, Yalcin S, Mungamuri SK, et al. FOXO3-mTOR metabolic cooperation in the regulation of erythroid cell maturation and homeostasis. *Am J Hematol* 2014; 89(10): 954-963.
41. Pecoraro A, Troia A, Calzolari R, Scazzone C, Rigano P, Martorana A, et al. Efficacy of rapamycin as inducer of Hb F in primary erythroid cultures from sickle cell disease and  $\beta$ -thalassemia patients. *Hemoglobin* 2015; 39(4): 225-259.
42. Zhang Y, Paikari A, Sumazin P, Summarell CCG, Crosby JR, Boerwinkle E, et al. Metformin induces FOXO3-dependent fetal hemoglobin production in human primary erythroid cells. *Blood* 2018; 132(3): 321-333.
43. Cappellini MD, Porter JB, Viprakasit V, Taher ATJBr. A paradigm shift on beta-thalassaemia treatment: How will we manage this old disease with new therapies? *Blood Rev* 2018; 32(4): 300-311.
44. Suragani RN, Cawley SM, Li R, Wallner S, Alexander MJ, Mulivor AW, et al. Modified activin receptor IIB ligand trap mitigates ineffective erythropoiesis and disease complications in murine  $\beta$ -thalassemia. *Blood* 2014; 123(25): 3864-3872.
45. Li H, Rybicki AC, Suzuka SM, Von Bonsdorff L, Breuer W, Hall CB, et al. Transferrin therapy ameliorates disease in  $\beta$ -thalassemic mice. *Nat med* 2010; 16(2): 177-182.
46. Lam M, Carmichael AR, Griffiths HRJPo. An aqueous extract of *Fagonia cretica* induces DNA damage, cell cycle arrest and apoptosis in breast cancer cells via FOXO3a and p53 expression. *PloS One* 2012; 7(6): e40152-e40159.
47. Humphreys JV. The management of sickle cell disease in a primary care

- setting. *J Family Med Prim Care* 2012 Jan; 1(1): 56-58.
48. Jacoby E, Chen A, Fuchs E, Jones R, Luznik L, Symons H. High dose cyclophosphamide for Graft-versus-Host disease prophylaxis following hematopoietic stem cell transplantation for pediatric hematologic malignancies. *Pediatr Blood Canc* 2013; 60-63.
49. Makis A, Hatzimichael E, Papassotiriou I, Voskaridou E. 2017 Clinical trials update in new treatments of  $\beta$ -thalassemia. *Am J Hematol* 2016; 91(11): 1135-1145.
50. Payen E, Leboulch P. Advances in stem cell transplantation and gene therapy in the  $\beta$ -hemoglobinopathies. *Hematology Am Soc Hematol Educ Program* 2012; 2012(1): 276-283.
51. Davis R, Gurusurthy A, Hossain MA, Gunn EM, Bungert J. Engineering globin gene expression. *Mol Ther Methods Clin Dev* 2018; 15(12): 102-110.
52. 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(4): 295-304.
53. Cavazzana M, Mavilio F. Gene therapy for hemoglobinopathies. *Hum Gene Ther* 2018; 29(10): 1106-1113.
54. Schuessler-Lenz M, Enzmann H, Vamvakas S. Regulators' Advice Can Make a Difference: European Medicines Agency Approval of Zynteglo for Beta Thalassemia. *Clin Pharmacol Ther* 2020; 107(3): 492-494.
55. Yu K-R, Natanson H, Dunbar CE. Gene editing of human hematopoietic stem and progenitor cells: promise and potential hurdles. *Hum gene Ther* 2016; 27(10): 729-740.
56. Walsh RM, Hochedlinger K. A variant CRISPR-Cas9 system adds versatility to genome engineering. *Proc Natl Acad Sci U S A* 2013; 110(39): 15514-15515.
57. Chandrasegaran S, Carroll D. Origins of programmable nucleases for genome engineering. *J Mol Biol* 2016; 428(5): 963-989.
58. Doudna JA, Charpentier E. The new frontier of genome engineering with CRISPR-Cas9. *Science* 2014; 346(6213): 1077-1088.
59. Eid A, Alshareef S, Mahfouz MM. CRISPR base editors: genome editing without double-stranded breaks. *Biochem J* 2018; 475(11): 1955-1964.
60. Ye L, Wang J, Tan Y, Beyer AI, Xie F, Muench MO, et al. Genome editing using CRISPR-Cas9 to create the HPFH genotype in HSPCs: An approach for treating sickle cell disease and  $\beta$ -thalassemia. *Proc Natl Acad Sci U S A* 2016; 113(38): 10661-10665.
61. Cui S, Kolodziej KE, Obara N, Amaral-Psarris A, Demmers J, Shi L, et al. Nuclear receptors TR2 and TR4 recruit multiple epigenetic transcriptional corepressors that associate specifically with the embryonic  $\beta$ -type globin promoters in differentiated adult erythroid cells. *Mol Cell Biol* 2011; 31(16): 3298-3311.
62. Li C, Psatha N, Sova P, Gil S, Wang H, Kim J, et al. Reactivation of  $\gamma$ -globin in adult  $\beta$ -YAC mice after ex vivo and in vivo hematopoietic stem cell genome editing. *Blood* 2018; 131(26): 2915-2928.
63. Canver MC, Smith EC, Sher F, Pinello L, Sanjana NE, Shalem O, et al. BCL11A enhancer dissection by Cas9-mediated in situ saturating mutagenesis. *Nature* 2015; 527(7577): 192-197.
64. Chang K-H, Smith SE, Sullivan T, Chen K, Zhou Q, West JA, et al. Long-term engraftment and fetal globin induction upon BCL11A gene editing in bone-marrow-derived CD34+ hematopoietic stem and progenitor cells. *Mol Ther Methods Clin Dev* 2017; 4: 137-148.
65. Basak A, Sankaran VG. Regulation of the fetal hemoglobin silencing factor BCL11A. *Ann N Y Acad Sci* 2016; 1368(1): 25-30.

66. Xu J, Sankaran VG, Ni M, Menne TF, Puram RV, Kim W, et al. Transcriptional silencing of  $\gamma$ -globin by BCL11A involves long-range interactions and cooperation with SOX6. *Genes dev* 2010; 24(8): 783-798.
67. Martyn GE, Wienert B, Yang L, Shah M, Norton LJ, Burdach J, et al. Natural regulatory mutations elevate the fetal globin gene via disruption of BCL11A or ZBTB7A binding. *Nat genet* 2018; 50(4): 498-503.
68. Psatha N, Reik A, Phelps S, Zhou Y, Dalas D, Yannaki E, et al. Disruption of the BCL11A erythroid enhancer reactivates fetal hemoglobin in erythroid cells of patients with  $\beta$ -thalassemia major. *Mol Ther Methods Clin Dev* 2018; 10: 313-326.
69. Wilber A, Tschulena U, Hargrove PW, Kim Y-S, Persons DA, Barbas CF, et al. A zinc-finger transcriptional activator designed to interact with the  $\gamma$ -globin gene promoters enhances fetal hemoglobin production in primary human adult erythroblasts. *Blood* 2010; 115(15): 3033-3041.
70. Love PE, Warzecha C, Li L. Ldb1 complexes: the new master regulators of erythroid gene transcription. *Trends Genet* 2014; 30(1): 1-9.
71. Deng W, Rupon JW, Krivega I, Breda L, Motta I, Jahn KS, et al. Reactivation of developmentally silenced globin genes by forced chromatin looping. *Cell* 2014; 158(4): 849-860.
72. Wang S, Chim B, Su Y, Khil P, Wong M, Wang X, et al. Enhancement of LIN28B-induced hematopoietic reprogramming by IGF2BP3. *Genes Dev* 2019; 33(15): 1048-1068.
73. Lulli V, Romania P, Morsilli O, Cianciulli P, Gabbianelli M, Testa U, et al. MicroRNA-486-3p regulates  $\gamma$ -globin expression in human erythroid cells by directly modulating BCL11A. *PloS one* 2013; 8(4): e60436.
74. Chateauvieux S, Grigorakaki C, Morceau F, Dicato M, Diederich M. Erythropoietin, erythropoiesis and beyond. *Biochem Pharmacol* 2011; 82(10): 1291-1303.
75. Chasis JA. Erythroblastic islands: specialized microenvironmental niches for erythropoiesis. *curr opin hematol* 2006; 13(3): 137-141.
76. Taher AT, Karakas Z, Cassinerio E, Siritanaratkul N, Kattamis A, Maggio A, et al. Efficacy and safety of ruxolitinib in regularly transfused patients with thalassemia: results from a phase 2a study. *Blood* 2018; 131(2): 263-265.
77. Gozzelino R, Arosio P. Iron homeostasis in health and disease. *Int J Mol Sci* 2016; 17(1): 130-135.
78. Gardenghi S, Ramos P, Marongiu MF, Melchiori L, Breda L, Guy E, et al. Hepcidin as a therapeutic tool to limit iron overload and improve anemia in  $\beta$ -thalassemic mice. *J Clin Invest* 2010; 120(12): 4466-4477.
79. Li H, Choesang T, Bao W, Chen H, Feola M, Garcia-Santos D, et al. Decreasing TfR1 expression reverses anemia and hepcidin suppression in  $\beta$ -thalassemic mice. *Blood* 2017; 129(11): 1514-1526.
80. Casu C, Oikonomidou PR, Chen H, Nandi V, Ginzburg Y, Prasad P, et al. Minihepcidin peptides as disease modifiers in mice affected by  $\beta$ -thalassemia and polycythemia vera. *Blood* 2016; 128(2): 265-276.
81. Guo S, Casu C, Gardenghi S, Booten S, Aghajan M, Peralta R, et al. Reducing TMPRSS6 ameliorates hemochromatosis and  $\beta$ -thalassemia in mice. *J Clin Invest* 2013; 123(4): 1531-1541.
82. Sauntharajah YJh. Targeting sickle cell disease root-cause pathophysiology with small molecules. *Haematologica* 2019; 104(9): 1720-1730.
83. Rivella S, Rachmilewitz E. Future alternative therapies for  $\beta$ -thalassemia. *Expert Rev Hematol* 2009; 2(6): 685-697.
84. Nemeth E, Ganz T. The role of hepcidin in iron metabolism. *Acta Haematol* 2009; 122(2-3): 78-86.