Current Practice

Management of Thalassaemia

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Sri Lanka Journal of Child Health, 2018; **47**(2): 159-165
DOI: http://dx.doi.org/10.4038/sljch.v47i2.8484
(Key words: Thalassaemia, management)

Introduction

Thalassaemia is a common globally prevalent monogenic disorder with an incidence of around 70,000 new cases per annum[1]. It is particularly common in the traditional 'thalassaemia belt' extending from the Mediterranean region via sub-Saharan Africa and Middle East to South and South-East Asia[2]. Prevalence of thalassaemia in Sri Lanka is high in comparison to many other countries in the region[3]. A recent island-wide survey revealed that there are approximately 2000 patients with severe thalassaemia in Sri Lanka and it is estimated that nearly 80 new patients are born each year[4]. Although many of these births occur in the North Western province and North Central province, no part of Sri Lanka can be considered as 'thalassaemia free'[5].

Thalassaemia was first described by an American paediatrician, Thomas B. Cooley, almost a century ago, in a paper which characterised five children with anaemia, splenomegaly and peculiar bone changes[6]. The pathophysiology and molecular basis of thalassaemia was unrevealed during the latter half of the 20th century and in fact, it is one of the earliest gene disorders to be characterised at the molecular level[7]. Despite this, the progress in management of thalassaemia has been extremely slow and the current standard of care is limited to supportive treatment in the majority[8]. However, during the last decade, significant advances have been made in many treatment modalities, genetic based therapies in particular. The aim of this paper is to discuss current standard of care and curative therapies and to provide an overview of emerging therapies for thalassaemia.

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The author declares that there are no conflicts of interest
Personal funding was used for the project.

Molecular basis

Thalassaemia arises from mutations in human globin genes encoding for α- and β-globin polypeptide chains of haemoglobin[9]. Two α- and 2 β-globin chains, each conjugated with an iron containing haem moiety, form adult haemoglobin (HbA). Molecular defects of thalassaemia lead to either reduced or absent production of β-globin chains resulting in β-thalassemia or defective synthesis of α-globin leading to α-thalassemia. In the Sri Lankan context, the vast majority of symptomatic and clinically significant thalassaemia is due to reduced production of β-globin chains and therefore this paper concentrates primarily on management of β-thalassaemia.

Beta-thalassaemia is an autosomal recessively inherited disease and over 250 mutations have been identified so far (complete list is found at http://globin.cse.psu.edu/hbvar/)[10]. The mutations are categorized into β^0-, β^+- and β^{++}-thalassemia alleles according to the degree of reduction of the β-globin chain output. Mutations that completely abolish the production of β-globin are known as β^0-thalassemia (severe) whilst mutations which result in milder reduction in β-globin are known as β^+-thalassemia. A group of mutations which are labelled as β^{++}-thalassemia results in only subtle effects on the β-globin chain synthesis[11]. Furthermore, a point mutation at codon 26 of the β-globin gene results in a structurally abnormal haemoglobin E ($\alpha_2\beta^E_2$) which also produces the phenotype of β-thalassemia. Inheritance of a β-thalassaemia allele from one parent and the HbE allele from the other parent gives rise to HbE/β-thalassaemia which accounts for nearly half of the patients with β-thalassaemia worldwide[12].

Clinical classification

Despite being a typical monogenic disorder, β-thalassemia exhibits a remarkable clinical heterogeneity with a broad spectrum of disease severity[13]. Individuals having a heterozygous mutation in β-globin gene (β-thalassemia trait) are asymptomatic, have minor haematological abnormalities and will not require blood transfusions. Conversely, patients at the severe end of the spectrum require regular life-long blood transfusions and are known as transfusion-dependent thalassaemia (TDT). These patients have

homozygous β-thalassemia mutations (β-thalassemia major) or have severe HbE/β-thalassaemia. Another substantial proportion of patients have an intermediate clinical phenotype with requirements for occasional blood transfusions. This subset is known as non-transfusion dependent thalassaemia (NTDT) which includes patients previously referred to as β-thalassemia intermedia and mild to moderate HbE/β-thalassaemia[10].

Diagnosis

Most patients with thalassaemia are diagnosed before two years of age. Patients with TDT present around 6-months of age with pallor, irritability, poor weight gain, abdominal distension and hepatosplenomegaly. Full blood counts show low haemoglobin (Hb), mean corpuscular volume and mean corpuscular haemoglobin. Peripheral blood smears demonstrate microcytes, poikilocytes, hypochromia, anisocytosis, and nucleated red cells[14]. Diagnosis of β-thalassaemia is confirmed by qualitative and quantitative Hb analysis, either by high-performance liquid chromatography or capillary electrophoresis. In β^0/β^0 thalassemia, HbF is 95–98%, HbA_2 is 2–5% and there is no HbA. In β^0/β^+- or β^+/β^+-thalassemia, HbA is 10–30%, HbF is 70–90%, and HbA_2 is 2–5%[15]. In HbE/β-thalassaemia, HbE constitutes between 30% and 70% of the Hb with the remainder being HbF. Depending on the nature of the associated β-thalassaemia mutation, variable levels of HbA can be found[12].

Current treatment options

Upon diagnosis, patients with thalassaemia should be referred to a specialist paediatrician who has experience in managing patients with thalassaemia. The current management options for thalassaemia include blood transfusion, iron chelation, splenectomy and haematopoietic stem cell transplantation.

Blood transfusion

Mainstay of thalassaemia management is blood transfusion, which aims to promote normal growth and physical activity and adequately suppress bone marrow activity whilst minimizing transfusion related iron accumulation. Transfusion protocol and frequency depend on whether the patient has TDT or NTDT which needs to be carefully decided during the first few years of treatment. However, this categorisation requires frequent revisiting throughout life as patients can move from TDT to NTDT and vice-versa due to variations in clinical management and changes in disease modifiers[16].

Before commencing the first blood transfusion, it is essential to establish an accurate diagnosis and to perform extended phenotyping of red blood cell (RBC) antigens (minor blood groups) to minimise future problems. Patients with TDT require regular transfusions usually 2-5 weekly, to maintain a target pre-transfusion Hb level of 9–10.5g/dl[17]. The currently accepted protocol is to achieve a post-transfusion Hb level of 14g/dl by transfusing leuco-depleted (reduced to $<1 \times 10^6$ leucocytes per unit) packed RBC. In patients with repeated severe allergic reactions, washed leuco-depleted packed RBC can be transfused. All patients with thalassaemia should receive fresh blood which is less than 2 weeks old. The volume of blood to be transfused is calculated using the following formula[18]:

Volume (ml) of blood to be transfused = (14g/dl – pre-transfusion Hb) x weight (kg) x 3 / haematocrit of transfused blood

Patients with NTDT need not be transfused regularly. Baseline haemoglobin levels as low as 6-7g/dl are accepted in NTDT patients and the frequency and need for transfusions should be decided individually[19]. Indications for transfusion in NTDT patients are not clearly defined but include growth failure, reduced exercise tolerance, poor quality of life and rapidly enlarging spleen (>3cm/year). Transfusion is also indicated during times of anticipated acute stress or low haemoglobin like infection or surgery[17]. Haemoglobin level per se is not considered as an indication for transfusion, unless it is very low (Hb <5 g/dl)[19].

Iron overload and chelation

Iron overload is an inevitable complication of thalassaemia. A unit of transfused packed RBC contains approximately 200–250 mg of iron and as humans essentially do not have a mechanism to excrete iron from the body, transfusional iron gets accumulated. Additionally, increased intestinal absorption of iron due to suppression of hepcidin secretion from the liver by increased red cell turnover in patients with thalassaemia contributes to iron overload[20]. Serum ferritin is the most widely used marker to assess iron overload and is measured 3-monthly in thalassaemia patients. In patients with TDT, serum ferritin above 1000µg/l is an indication to start iron chelation and serum ferritin above 2500µg/l is associated with increased risk of cardiac and endocrine disease. In patients with NTDT, iron chelation is indicated when serum ferritin rises above 800µg/l[19].

Use of magnetic resonance imaging (MRI) to non-invasively assess liver and myocardial iron concentration is an important advance in thalassaemia care. Liver iron concentration can be measured both by T2* and R2 (also known as FerriScan®) techniques whereas, cardiac iron is assessed by T2* MRI. Cardiac T2* values <20ms are indicative of declining left ventricular function whilst values below 10ms are associated with heart

failure and very high mortality[16]. However, due to limited availability of MRI based techniques, serum ferritin still remains the most widely used investigation to assess iron overload in many low- and middle- income countries like Sri Lanka.

Three iron chelators are currently available to treat iron overload in thalassaemia patients, viz. desferrioxamine, deferasirox and deferiprone (Table 1).

Table 1: ***Comparison of currently available iron chelators***

	Desferrioxamine	**Deferasirox**	**Deferiprone**
Route	subcutaneous or intravenous	oral	oral
Frequency	8-12hr infusion, 5-7 days /week	once daily	three times daily
Dose (mg/kg/day)	30-60	20-40 (TDT); 5-20 (NTDT)	75-100
Route of iron excretion	urinary and faecal	faecal	urinary
Main adverse effects	ocular and auditory symptoms, retardation of bone growth, local reactions, allergy	GI symptoms, increased creatinine, increased hepatic enzymes	GI symptoms, arthralgia, agranulocytosis/ neutropenia
Advantages	extensive experience, low cost, better availability	better compliance	chelates cardiac iron more efficiently
Disadvantages	poor compliance	limited safety data	not recommended as monotherapy

TDT: transfusion-dependent thalassaemia, NTDT: non-transfusion dependent thalassaemia, GI: gastrointestinal

Desferrioxamine was the firstly developed and most efficacious iron chelator for many years. However, unavailability of an oral dosage form and the need for subcutaneous infusion for long hours markedly limited its use. Since its development, deferasirox, an oral chelator with a similar efficacy to desferrioxamine, has been the first line iron chelator for iron overload in thalassaemia patients in many countries worldwide. Our current practice is to use deferasirox as first line iron chelator in children above two years. Desferrioxamine is used as first line in children less than 2 years, with contraindications for deferasirox and as an emergency treatment for acute cardiac failure due to cardiomyopathy. Deferiprone has a higher chelating ability of myocardial iron compared to other drugs but is not recommended as monotherapy. Combination of deferasirox and desferrioxamine is used when a single chelator is unable to control iron overload and combination of desferrioxamine and deferiprone is used especially when cardiac iron chelation becomes a priority. However, the combination of deferasirox and deferiprone has not been well studied[18].

Splenectomy

Splenectomy was traditionally performed in most patients with TDT and NTDT as an adjunct to transfusion therapy. However, this procedure is not routinely performed at present due to reports of adverse events caused by splenectomy which include increased risk of post-splenectomy sepsis, venous thrombosis, pulmonary hypertension, leg ulcers and silent strokes[16]. Current recommendations for splenectomy are limited to patients with hypersplenism and clinically symptomatic splenomegaly. However, splenectomy should also be considered on an individual basis in patients with very high transfusion requirements, particularly when the annual transfusion requirement rises above 200-250ml/kg/year[17,21].

Screening for complications

Thalassaemia is a life-long, progressive disease and the current standard of care only provides supportive treatment for the majority of patients. Patients with thalassaemia thus develop long-term complications related to chronic anaemia, multiple blood transfusions, iron overload and adverse effects of iron chelator medication. Therefore, continuous surveillance of these complications is essential in providing optimal care for patients with thalassaemia. Common complications of thalassaemia and the recommended approach for screening are provided in table 2[18]. Management of individual complications is outside the scope of this article.

Table 2: ***Long-term complications of thalassaemia and approach for screening***

Complication	Parameter to monitor	Frequency (months)				
		1	3	6	12	24
Growth failure	Weight		X			
Short stature	Height/height velocity			X		
Delayed puberty	Tanner staging (from 10yrs)			X		
Dental malocclusion	Dental assessment				X	
Hypersplenism	Full blood count	X				
Iron overload	Serum ferritin		X			
Hepatic dysfunction	AST/ALT/bilirubin/albumin		X			
	Abdominal ultrasonography				X	
	Liver MRI (from 8yrs)				X	
Transfusion transmitted infections	Hepatitis A, B and C screen				X	
Renal dysfunction	Creatinine		X			
	Urinalysis			X		
Cardiomyopathy	Electrocardiography				X	
	Echocardiography				X	
	Cardiac T2* MRI (from 8yrs)				X	
Hypothyroidism	TSH/T4/T3 (from 5yrs)				X	
Hypoparathyroidism	Calcium (from 5yrs)		X			
	Parathormone level (from 5yrs)				X	
Diabetes mellitus	Fasting blood glucose (from 5yrs)		X			
	Glucose tolerance test (from 10yrs)				X	
Osteoporosis	Vitamin D level			X		
	DEXA scan					X
Adverse effects of drugs	Creatinine (DFX)	X				
	ALT (DFX/DFP)	X				
	Leucocyte count (DFP)	X				
	Ophthalmology evaluation (DFO/DFX/DFP)				X	
	Audiology evaluation (DFO/DFP/DFX)				X	

AST: Aspartate transaminase, ALT: Alanine transaminase, MRI: Magnetic resonance imaging, TSH: Thyroid stimulating hormone, T4: Thyroxine, T3: Triiodothyronine, DFO: Desferrioxamine, DFP: Deferiprone, DFX: Deferasirox

Haematopoietic stem cell transplantation (HSCT)

Allogenic HSCT is the only available cure for thalassaemia. The first HSCT for thalassaemia was performed in 1982 and over 3000 HSCT have been performed for thalassaemia up to now. Best outcomes with overall survival of 90% and disease-free survival of over 80%, are reported when transplanting haematopoietic stem cells (HSC) from HLA-matched sibling donors[22]. However, only about 10% of thalassaemia patients are fortunate to have HLA-matched sibling donors which limits the usefulness of this treatment option. Use of HLA matched-unrelated donors or umbilical cord blood as the HSC source for patients who do not have matched-siblings has been practised in many centres with overall survival rates ranging from 60% to 65%. Mortality related to transplant conditioning, graft-versus-host disease and graft failure continues to limit the acceptability of this treatment method. Currently HSCT is routinely indicated only in patients who are transfusion dependent and have well-matched sibling donors. Outcome of HSCT is greatest if it is performed before 14-years of age and iron-related complications have developed[23].

Emerging therapies

Several new therapies, both pharmacological and gene-based are currently being investigated for thalassaemia. Most of these aim to restore globin chain imbalance in thalassaemia RBCs. Induction of γ-globin with an aim to increase fetal haemoglobin production using pharmacological agents has been tried for several years[24]. Despite numerous drugs having reached clinical trials, none has been efficacious enough to be recommended for routine clinical use[25]. Hydroxyurea, which has produced best results thus far is widely used to increase fetal haemoglobin production to prevent sickling crisis in patients with sickle cell disease but has not been shown to reduce the need for blood transfusion in patients with thalassaemia[26]. An alternative approach that has reached clinical trials is improving ineffective erythropoiesis by inhibiting its mediators using ruxolitinib (JAK2 inhibitor) or luspatercept (activin type IIB receptor ligand trap)[17]. Another

approach which is still in the pre-clinical stage is pharmacological down regulation of α-globin with an aim to improve globin chain balance[27,28]. Use of epigenetic drugs has been proposed for this purpose[29].

Gene therapy has been on the horizon for many years as a cure for thalassaemia. Contrary to initial promise the progress in the field has been painfully slow. The work flow of gene therapy involves harvesting of HSCs from patients with thalassaemia, insertion of normal β-globin gene using viral vectors in vitro, and transplanting the HSC with normal β-globin gene back to the patient as an autologous HSCT[30]. First gene therapy trial for thalassaemia was started in 2007 and thus far approximately ten patients have been treated[31]. Although transfusion independence in a patient who underwent gene therapy was reported in 2010, success of the approach has been limited due to poor efficacy in bone marrow harvesting, gene transfer and normal gene expression as well as due to the oncogenic potential of the procedure[32].

Recently, genome editing, a new form of gene therapy, has shown promise in pre-clinical studies. Contrary to traditional gene therapy, genome editing utilises programmable nucleases to create an edit in a pre-determined target site in the human genome[33]. Genome editing has been used to upregulate γ-globin by mutating the γ-globin suppressor *BCL11A* gene or to downregulate α-globin by mutating its enhancers[34,35]. Both these approaches have shown promise in *in vitro* studies but require further validation before progressing to clinical trials.

Summary

As a result of the widespread availability of facilities for safe blood transfusions and the advent of efficacious iron chelators, supportive care of patients with thalassaemia has shown remarkable improvement over the past decade. Average life expectancy has risen considerably and a large proportion of patients with thalassaemia now lives beyond 40 years. Better chelation and availability of oral iron chelators have greatly improved the quality of life of these patients and many live near normal lives. Despite having several limitations, cure of thalassaemia is currently possible through HSCT in patients who have well-matched donors. There are several novel developments in the thalassaemia field at therapeutic level and advances in gene therapy or genome editing will alleviate the need for HLA-matched donors for HSCT and may provide cures for all thalassaemia patients in the future.

References

1. Weatherall DJ. The inherited diseases of haemoglobin are an emerging global health burden. *Blood* 2010; **115**(22):4331-6. https://doi.org/10.1182/blood-2010-01-251348 PMid: 20233970 PMCid: PMC2881491

2. Weatherall DJ. The challenge of haemoglobinopathies in resource-poor countries. *British Journal of Haematology* 2011; **154**(6):736-44. https://doi.org/10.1111/j.1365-2141.2011.08742.x PMid: 21726207

3. de Silva S, Fisher CA, Premawardhena A, Lamabadusuriya SP, Peto TE, Perera G, et al. Thalassaemia in Sri Lanka: implications for the future health burden of Asian populations. Sri Lanka Thalassaemia Study Group. *Lancet* 2000; **355**(9206):786-91. https://doi.org/10.1016/S0140-6736(99)08246-X

4. Premawardhana AP, Mudiyanse RM, Jifri MN, Nelumdeniya UBP, Silva DPSI, Nizri AHM, et al. An island-wide hospital based epidermiological survey of haemoglobinopatthies and an assessment of standards of care in 23 centres. *Ceylon Medical Journal* 2017; **62**(suppl. 1):73.

5. Mettananda S, de Silva DG. Anaemia in children: are we using the correct prevention strategies? *Ceylon Medical Journal* 2017; **62**(2):73-6. https://doi.org/10.4038/cmj.v62i2.8469 PMid: 28697539

6. Cooley TB, Lee P. A series of cases of splenomegaly in children, with anaemia and peculiar bone changes. *Transactions of the American Pediatric Society* 1925; **37**:29-30.

7. Weatherall DJ, Clegg JB. The thalassaemia syndromes. 4 ed. Oxford: Blackwell Science; 2001. https://doi.org/10.1002/9780470696705

8. Mettananda S. Thalassaemia: In a quest towards an ultimate cure. *Sri Lanka Journal of Child Health* 2017; **46**(3):203-10. https://doi.org/10.4038/sljch.v46i3.8318

9. Higgs DR, Engel JD, Stamatoyannopoulos G. Thalassaemia. *Lancet* 2012; **379**(9813):373-83. https://doi.org/10.1016/S0140-6736(11)60283-3

10. Mettananda S, Higgs DR. Molecular basis and genetic modifiers of thalassaemia. *Hematology/Oncology Clinics of North America* 2018; **32**(2):177-91. https://doi.org/10.1016/j.hoc.2017.11.003 PMid: 29458725

11. Thein SL. Molecular basis of beta thalassemia and potential therapeutic targets. Blood cells, molecules & diseases. 2017. https://doi.org/10.1016/j.bcmd.2017.06.001 PMid: 28651846

12. Fucharoen S, Weatherall DJ. The haemoglobin E thalassemias. *Cold Spring Harbor Perspectives in Medicine* 2012; **2**(8). https://doi.org/10.1101/cshperspect.a011734 PMid: 22908199 PMCid: PMC3405827

13. Musallam KM, Rivella S, Vichinsky E, Rachmilewitz EA. Non-transfusion-dependent thalassemias. *Haematologica* 2013; **98**(6):833-44. https://doi.org/10.3324/haematol.2012.066845 PMid: 23729725 PMCid: PMC3669437

14. Origa R. beta-Thalassemia. *Genetics in Medicine : Official Journal of the American College of Medical Genetics* 2017; **19**(6):609-19. https://doi.org/10.1038/gim.2016.173 PMid: 27811859

15. Cao A, Galanello R. Beta-thalassemia. *Genetics in Medicine : Official Journal of the American College of Medical Genetics* 2010; **12**(2):61-76. https://doi.org/10.1097/GIM.0b013e3181cd68ed PMid: 20098328

16. Taher AT, Weatherall DJ, Cappellini MD. Thalassaemia. *Lancet* 2018; **391**(10116):155-67. https://doi.org/10.1016/S0140-6736(17)31822-6

17. Cappellini MD, Porter JB, Viprakasit V, Taher AT. A paradigm shift on beta-thalassaemia treatment: How will we manage this old disease with new therapies? *Blood Reviews* 2018. https://doi.org/10.1016/j.blre.2018.02.001 PMid: 29455932

18. Cappellini MD, Cohen A, Porter J, Taher A, Viprakasit V. Guidelines for the management of transfusion dependent thalassaemia (TDT) 3rd Edition ed. Cappellini MD, Cohen A, Porter J, Taher A, Viprakasit V, editors. Cyprus: Thalassaemia International Federation; 2014.

19. Taher A, Vichinsky E, Musallam K, Cappellini MD, Viprakasit V. Guidelines for the management of non transfusion dependent thalassaemia (NTDT) Cyprus: Thalassaemia International Federation; 2013.

20. Bou-Fakhredin R, Bazarbachi AH, Chaya B, Sleiman J, Cappellini MD, Taher AT. Iron overload and chelation therapy in non-transfusion dependent thalassaemia. *International Journal of Molecular Science* 2017; **18**(12). https://doi.org/10.3390/ijms18122778 PMid: 29261151 PMCid: PMC5751376

21. Musallam KM, Angastiniotis M, Eleftheriou A, Porter JB. Cross-talk between available guidelines for the management of patients with beta-thalassemia major. *Acta Haematologica* 2013; **130**(2):64-73. https://doi.org/10.1159/000345734 PMid: 23485589

22. Baronciani D, Angelucci E, Potschger U, Gaziev J, Yesilipek A, Zecca M, et al. Hemopoietic stem cell transplantation in thalassemia: a report from the European Society for Blood and Bone Marrow Transplantation Hemoglobinopathy Registry, 2000-2010. *Bone marrow transplantation* 2016; **51**(4):536-41. https://doi.org/10.1038/bmt.2015.293 PMid: 26752139

23. Angelucci E, Matthes-Martin S, Baronciani D, Bernaudin F, Bonanomi S, Cappellini MD, 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**(5):811-20. https://doi.org/10.3324/haematol.2013.099747 PMid: 24790059 PMCid: PMC4008115

24. Musallam KM, Taher AT, Cappellini MD, Sankaran VG. Clinical experience with fetal hemoglobin induction therapy in patients with beta-thalassemia. *Blood* 2013; **121**(12):2199-212; quiz 372. https://doi.org/10.1182/blood-2012-10-408021 PMid: 23315167

25. Okam MM, Esrick EB, Mandell E, Campigotto F, Neuberg DS, Ebert BL. Phase 1/2 trial of vorinostat in patients with sickle cell disease who have not benefitted from hydroxyurea. *Blood* 2015; **125**(23):3668-9. https://doi.org/10.1182/blood-2015-03-635391 PMid: 26045597 PMCid: PMC4458806

26. Foong WC, Ho JJ, Loh CK, Viprakasit V. Hydroxyurea for reducing blood transfusion in non-transfusion dependent beta thalassaemias. *The Cochrane Database of Systematic Reviews* 2016; **10**:CD011579. https://doi.org/10.1002/14651858.CD011579.pub2

27. Mettananda S, Gibbons RJ, Higgs DR. alpha-globin as a molecular target in treatment of beta-thalassemia. *Blood* 2015. https://doi.org/10.1182/blood-2015-03-633594 PMid: 25869286 PMCid: PMC4497969

28. Mettananda S, Gibbons RJ, Higgs DR. Understanding alpha-globin gene regulation and implications for the treatment of beta-thalassemia. *Annals of the New York Academy of Sciences* 2016; **1368**(1):16-24. https://doi.org/10.1111/nyas.12988 PMid: 26695885

29. Mettananda S, Fisher CA, Sloane-Stanley JA, Taylor S, Oppermann U, Gibbons RJ, et al. Selective silencing of alpha-globin by the histone demethylase inhibitor IOX1: a potentially new pathway for treatment of beta-thalassemia. *Haematologica* 2017; **102**(3):e80-e4. https://doi.org/10.3324/haematol.2016.155655 PMid: 27810991 PMCid: PMC5394973

30. Cavazzana M, Antoniani C, Miccio A. Gene Therapy for beta-Hemoglobinopathies. *Molecular Therapy: the Journal of the American Society of Gene Therapy* 2017; **25**(5):1142-54. https://doi.org/10.1016/j.ymthe.2017.03.024 PMid: 28377044

31. Cavazzana-Calvo M, Payen E, Negre O, Wang G, Hehir K, Fusil F, et al. Transfusion independence and HMGA2 activation after gene therapy of human beta-thalassaemia. *Nature* 2010; **467**(7313):318-22. https://doi.org/10.1038/nature09328 PMid:20844535 PMCid:PMC3355472

32. Persons DA. Gene therapy: Targeting beta-thalassaemia. *Nature* 2010; **467**(7313):277-8. https://doi.org/10.1038/467277a PMid: 20844523

33. Ledford H. CRISPR, the disruptor. *Nature* 2015; **522**(7554):20-4. https://doi.org/10.1038/522020a PMid: 26040877

34. Mettananda S, Fisher CA, Hay D, Badat M, Quek L, Clark K, et al. Editing an alpha-globin enhancer in primary human hematopoietic stem cells as a treatment for beta-thalassemia. *Nature Communications* 2017; **8**(1):424. https://doi.org/10.1038/s41467-01700479-7 PMid: 28871148 PMCid: PMC5583283

35. 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):192-7 https://doi.org/10.1038/nature15521 PMid: 26375006 PMCid: PMC4644101