A Review On

"Thalassemia and Cell Based Gene Therapy (Zynteglo)"

[In the partial fulfilment of the requirements for the degree of Bachelor of Pharmacy (B. Pharm.)]

Submitted To

Department of Pharmacy

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Approval

This project paper, **"Review on Thalassemia and Cell Based Gene Therapy (Zynteglo)"** submitted to the Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University, has been accepted as satisfactory for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy and approved as to its style and contents.

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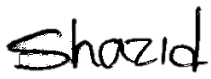

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ABSTRACT

Thalassemia is a group of inherited blood disorders characterized by decreased or absent production of one or more of the globin chains that make up hemoglobin. It is a major public health problem, particularly in the Mediterranean region, Middle East, and Southeast Asia. While blood transfusions and iron chelation therapy have improved the life expectancy of patients with thalassemia, they are not curative and have significant limitations. Cell-based gene therapy is a promising approach for the treatment of thalassemia. One such therapy, Zynteglo, involves the ex vivo transduction of autologous hematopoietic stem cells with a lentiviral vector carrying a functional copy of the β-globin gene. The modified cells are then infused back into the patient's bloodstream, where they differentiate into red blood cells that produce functional hemoglobin.

This review provides an overview of thalassemia and the current state of cell-based gene therapy for this condition, with a focus on Zynteglo. It discusses the safety profile of the therapy, as well as its potential limitations.

Keywords: Thalassemia, hemoglobin, gene therapy, zynteglo, prevention

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Chapter-1
Introduction

1. Introduction

1.1. Thalassemia

Thalassemias are a group of inherited blood illnesses that are characterised by decreased haemoglobin production. These abnormalities are handed down via families. Depending on the kind, the intensity of the symptoms might vary anywhere from none at all to a significant amount. The degree of anaemia may vary from very minor to very severe (low red blood cells or hemoglobin). Having anaemia is characterised by a number of symptoms, including feeling weary and having pale skin [1]. A yellowing of the skin, urine that is black in colour, difficulties with the bones, and an enlarged spleen are some more symptoms that may be present. It's possible that children may develop more slowly than planned.Thalassemias are conditions that are passed on from generation to generation [2]. There are two basic kinds of this illness, which are known as alpha thalassemia and beta thalassemia.The severity of alpha and beta thalassemia is dictated by the amount of genes for alpha or beta globin that are missing from the body. This holds true for both forms of the disease. In order to identify the illness, blood tests, such as a complete blood count, specialised haemoglobin tests, and genetic studies are often used [3-4]. A diagnosis is able to be established before to the birth of the child via the use of prenatal testing.The treatment is tailored to the patient's condition and level of severity and may entail the use of medicines [5-6]. As a rule, patients with a more severe form of the condition need more frequent blood transfusions, iron chelation, and folic acid therapy as part of their course of medical treatment [7]. Deferoxamine, deferasirox, or deferiprone are all excellent iron chelating agents that may be utilised in a therapy. You can choose whichever one works best for you. A transplant of bone marrow may sometimes be an effective form of therapy for certain conditions. Receiving transfusions carries with it the risk of developing a number blood problems, including an excess of iron, which raises the risk of developing disorders of the heart or liver, infections, and osteoporosis. If the spleen becomes excessively large, it may be essential to have surgery to remove it in order to prevent further complications [9]. Individuals diagnosed with thalassemia who do not respond well to blood transfusions have the choice of either taking hydroxyurea or thalidomide, and in certain instances, a combination of the two medications is also an option [10]. The Food and Drug Administration has given its approval to hydroxyurea as the only therapy for thalassemia. People who did not respond well to blood transfusions were often able to undergo the treatment of taking 10 mg/kg of hydroxyurea every day for a whole year. This was done in order to treat the

anaemia caused by the disease. The patients had significantly greater levels of hemoglobin as a consequence of this medicine, which was also typically well tolerated by the patients [11].

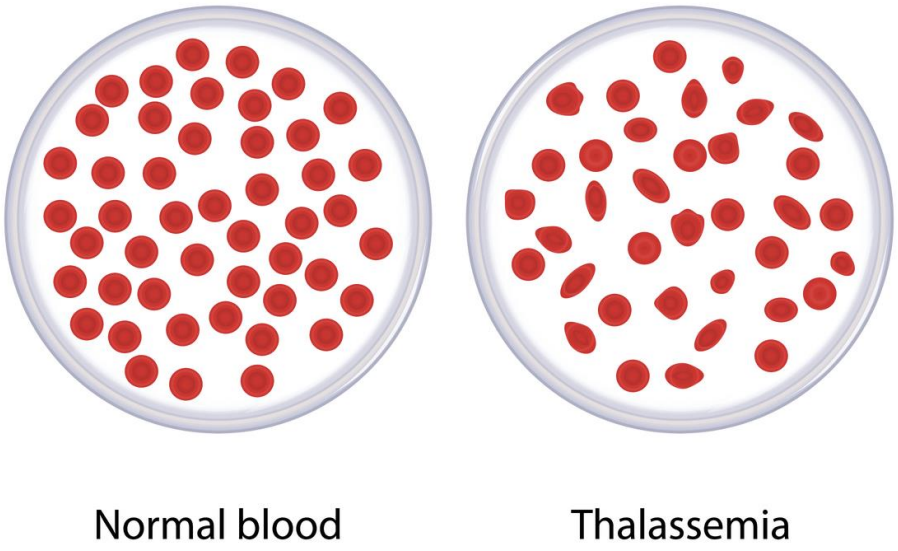

Fig 01: Thalassemia [News Medical:online]

Thalidomide is yet another example of a hemoglobin-inducer, despite the fact that it has not been tested in a clinical setting. Thalidomide was developed in the 1950s. The combination of thalidomide and hydroxyurea led to a significant rise in the haemoglobin levels of all of the test subjects, including those who were needed to have blood transfusions as well as those who were not required to receive such treatment [12].As of the year 2015, there were roughly 280 million people who were affected by thalassemia, with approximately 439,000 people suffering from a severe version of the disorder [13]. Those of Greek, Italian, Middle Eastern, South Asian, and African background have the greatest prevalence rates of this syndrome. Individuals of South Asian and African descent had the second highest prevalence rates.The frequency of disease is the same in both boys and females to the same extent [14]. In 2015, it was responsible for 16,800 fatalities, which is a considerable decline from the 36,000 deaths it caused in 1990. In 1990, the number of fatalities it caused was much higher [15]. Those who have milder types of thalassemia, which are similar to persons who have sickle-cell trait, have some protection against malaria. This protection is akin to having sickle-cell trait. This helps to explain why there are a disproportionate

number of persons with these types of thalassemia in regions of the world that are prone to malaria [16].

1.1.1 Signs and symptoms

Iron overload: Thalassemia patients have a higher risk of developing an iron overload in their systems, which may be caused by the illness itself or by the numerous blood transfusions they get. The endocrine system, which includes glands that generate hormones that govern activities throughout the body, may be harmed when an excessive amount of iron is consumed. This can also cause damage to the heart and the liver. The damage is distinguished by the presence of an excessive amount of iron deposits. In virtually all cases, individuals with beta-thalassemia will acquire potentially lethal quantities of iron if they do not get appropriate iron chelation treatment [17].

Infection: Those who have thalassemia have a greater chance of becoming sick from infections. This is true in particular in cases when the spleen has been removed [18].

Bone deformities: Thalassemia is known to cause the bone marrow to enlarge, which in turn leads the bones to widen. This may lead to aberrant bone structure, particularly in the face and skull, especially in children. The development of bone marrow may also cause bones to become fragile and thin, which increases the likelihood that they will break [19].

Enlarged spleen: The spleen plays an important role in the immune system by helping to combat infections and filtering out undesired material, such as blood cells that have become damaged or obsolete.

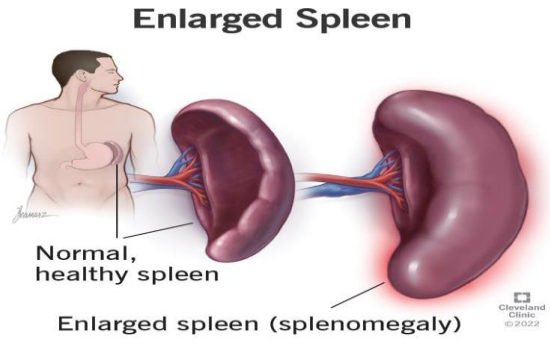

Fig 02: Enlarged spleen [Clevel and Clinic :online]

It is common for thalassemia to be accompanied with the breakdown of a significant number of red blood cells, and the process of eliminating these cells leads the spleen to grow in size. Splenomegaly may make anaemia worse, and it can also shorten the lifespan of red blood cells that have been transfused. When the spleen has become very enlarged, it may be necessary to remove it surgically [20].

Slowed growth rates: Anemia is one of the conditions that might cause a child's development to proceed at a more snail-like pace. Children who have thalassemia may also have a delay in the onset of puberty [21].

Heart problems: Problems with the heart Severe thalassemia may be linked with a variety of cardiac diseases, including congestive heart failure and irregular heart rhythms [22].

1.1.2. The Structural Biology of Hemoglobin

Hemoglobins are tetrameric proteins that are built from two pairs of globin chains in order to be found in persons who have immune systems that are functioning normally. Within of every pair of globin chains, there is one chain that has a structure that is analogous to an alpha-like structure and one chain that has a structure that is analogous to a beta-like structure. Each globin chain that makes up the protein has an iron-containing heme moiety linked to it somewhere along its length. This attachment may take place anywhere along the chain. The synthesis of alpha-like chains and beta-like chains, which are also known as non-alpha-like chains, are balanced inside a living species in such a manner that the ratio of the two types of chains is normally constant, and there is no excess of either form of chain [23]. Over the whole of the process of development, a significant level of control is taken over which particular alpha and beta-like chains are going to be included into Hb: The expression of embryonic Hbs starts as early as four to six weeks during the development of the embryo and continues until around the eighth week of gestation, when it is replaced by foetal Hb. This process begins as early as four to six weeks during the development of the embryo. During the development of the embryo, this process may begin as early as four to six weeks after fertilization [24-25].

The following elements contribute to the formation of embryonic Hbs:

- Hb Gower-1 is a kind of haemoglobin that is made by combining the components of two zeta globins and two epsilon globins (zeta and epsilon globins). (zeta and epsilon globins)

(zeta and epsilon globins) (zeta and epsilon globins) (zeta and epsilon globins) (zeta and Hb The globin variant known as Gower-2 is created by fusing together the constituent parts of two alpha globins and two epsilon globins.

- In addition to containing two gamma globins and two zeta globins, the structure of the haemoglobin known as Hb Portland also has two zeta globins.
- The production of foetal haemoglobin, commonly known as Hb F, begins during the ninth week of pregnancy and continues right up until the baby is born. In a baby that has reached full term, it is responsible for around 80 percent of the entire amount of haemoglobin that is present in the newborn's body. While its level begins to decrease during the first few months of life, by the time a healthy kid is in their early years, it makes up less than one percent of total Hb. This is despite the fact that its level starts to decrease during the first few months of life. In a youngster with aberrant development, it constitutes more than one percent of the child's total Hb. The 22 formula is what makes up Hb F. This formula consists of two alpha globins, two gamma globins, and a mixture of both of these globin types.
- Haemoglobin A, or adult haemoglobin, is the most prevalent form of haemoglobin found in babies and children older than six months. It is also usually referred to by its acronym, Hb A. In people who are otherwise healthy and do not have a hemoglobinopathy, it accounts for between 96 and 97% of the total Hb. It contains the formula 22, which shows that it is comprised of two alpha globins and two beta globins in equal quantities. This may be deduced from the fact that it has four globins in total.
- Hb A2 is a minor adult Hb that generally contributes for around 2.5-3.5% of total Hb and starts to form in the body at six months of age. Hb A2 is a little adult Hb. It is something that evolves over the course of a lifetime. It is made up of two alpha globins and two delta globins in proportions that are the same as those seen in other similar structures.

1.1.3 Cause

Autosomal recessive inheritance is the most common mode through which - and -thalassemia are transmitted from one generation to the next. There have been examples of dominantly inherited thalassemias that have been reported. The first of these instances was discovered in an Irish family. It was caused by two deletions in exon 3 consisting of 4 and 11 base pairs, which were interrupted by an insertion of 5 base pairs in the -globin gene. It is necessary for both of a child's parents to be

carriers of the disease for the child to have a chance of inheriting a form of the sickness that is transmitted by autosomal recessive inheritance. If the hemoglobinopathy characteristic is passed down from both the mother and the father, then there is a one in four chance that the child will be affected by the condition.The genes that are thought to have a role in thalassemia are the ones responsible for controlling the production of normal haemoglobin. The lungs are in charge of the process of oxygen being bound to haemoglobin, and the oxygen that has been bound will not be released by red cells until they reach peripheral organs like the liver. The mechanism through which haemoglobin simultaneously attaches to and releases oxygen is one that is absolutely essential to living things.

Evolution

- ✓ Even the existence of a single genetic mutation for thalassemia may confer protection against malaria, therefore having even one of these variations is likely to be beneficial [26].
- ✓ Those who have been found to have the heterozygous (carrier) type of beta-thalassemia have a lower chance of getting coronary heart disease. This is because carriers of the illness do not express the condition [27].

1.1.4 Pathophysiology

Hemoglobin A, which makes up the bulk of adult haemoglobin, is typically made up of four protein chains that are grouped as a heterotetramer. These chains are two -globin chains and two -globin chains. Patients with thalassemia have abnormalities in either the -globin chain or the -globin chain, which results in the generation of abnormal red blood cells.Depending on whether chain of the haemoglobin molecule is disrupted, there are many different types of thalassemia. In -thalassemias, there is a disruption in the manufacture of the -globin chain, while in -thalassemia, there is a disruption in the synthesis of the -globin chain [28]. On chromosome 11, a single gene is responsible for encoding the -globin chains, whereas on chromosome 16, two genes that are tightly related are responsible for doing the same thing [29]. Hence, in a healthy individual with two copies of each chromosome, two loci are responsible for encoding the chain, and four loci are responsible for encoding the chain. People of African or Asian heritage have a higher incidence of the deletion of one of the loci, which increases the likelihood that these individuals would acquire

-thalassemia. Africans are not the only people that have a high prevalence of beta-thalassemia; Greeks and Turks do as well.

Alpha-thalassemias

The -thalassemias are caused by mutations in the HBA1 [30] and HBA2 [31] genes, which are passed down in a Mendelian recessive manner. Since there are two gene loci, there are four possible alleles. Since there are two genetic loci for -globin, cells that are diploid have all four possible alleles. Four of the alleles have a maternal origin, whereas the other four have a paternal origin. There is a correlation between the severity of the illness and the number of afflicted -globin; alleles. The bigger the number, the more severe the symptoms of the disease will be [32]. Alpha-thalassemias lead to a reduction in the synthesis of alpha-globin; as a consequence, fewer alpha-globin chains are generated, which leads to an excess of chains in adults and an excess of chains in infants. The anomalous oxygen dissociation curves are a result of the abnormal formation of unstable tetramers (called haemoglobin H or HbH of 4 beta chains) by the excess beta chains. Those who are of African ancestry, Chinese descent, Middle Eastern heritage, or Southeast Asian origin are more likely to have alpha thalassemia than other persons [33].

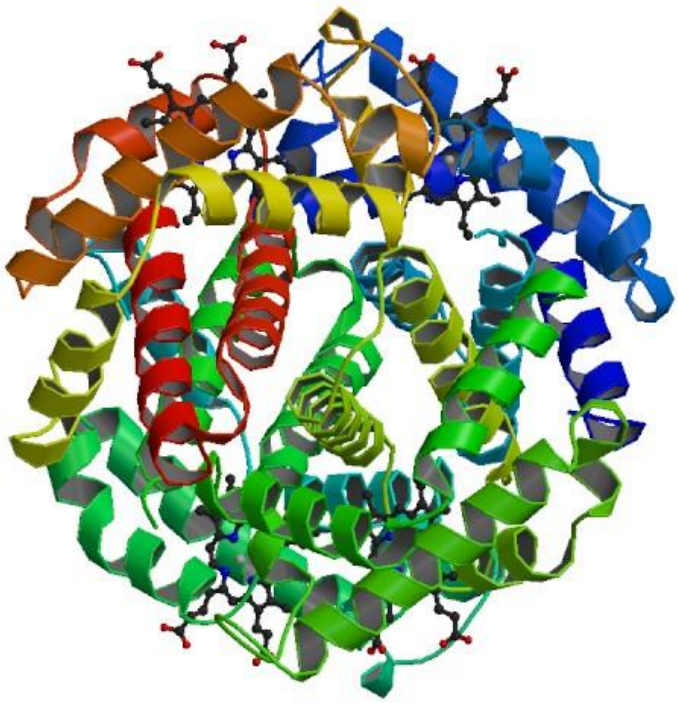

Fig 03: HBA1 [WikiDoc :online]

Beta-thalassemia

Autosomal recessive inheritance is also the mode of transmission for beta thalassemias, which are caused by mutations in the HBB gene located on chromosome 11. The severity of the condition may vary depending on the kind of mutation that has occurred as well as whether or not mutations have been found in each allele.When just a portion of a protein's function is lost due to a mutation, the allele is referred to as +, but when no functional protein is generated, it is referred to as o. Mutated alleles are denoted by one of these two designations.The clinical picture is determined by the states of both alleles, which are:thalassemia major, often known as Mediterranean anaemia or Cooley anaemia, is brought on by a genotype known as o/o. Since there are no functional chains formed, there is no possibility of haemoglobin A being constructed. -thalassemia intermedia is the most severe form of the disease and is brought on by either a +/o genotype or a +/+ genotype. thalassemia minor is characterised by the production of a little amount of haemoglobin A and is brought on by either an o/o or o/+ genotype. Since just one of the two globin alleles is mutated, synthesis of the chain is not severely affected, and patients may exhibit few symptoms as a result.Those who are of Mediterranean heritage are the most likely to be affected with beta thalassemia. Chinese people, people of various Asian origins, and Black Americans may be impacted to a lesser degree [34].

Delta-thalassemia

Around 3% of an adult's haemoglobin is composed of alpha and delta chains, in addition to the alpha and beta chains that are already present in the molecule. In the same way as beta thalassemia may be caused by mutations, delta thalassemia can also be caused by mutations in the gene that controls the production of delta chains [35-37].

1.1.5 Diagnosis

The procedure of diagnosing thalassemia might include a variety of tests, including a complete blood count, haemoglobin electrophoresis or high-performance liquid chromatography, and DNA analysis, to name just a few of the possibilities [38-39]. Since haemoglobin electrophoresis is difficult to come by in developing nations, the Mentzer index is sometimes used to diagnose thalassemia instead. While the results of this test are not definitive, they do increase the possibility

that thalassemia is present. The results of a complete blood count may be used to calculate the Mentzer index. This is something that can be quite helpful [40].

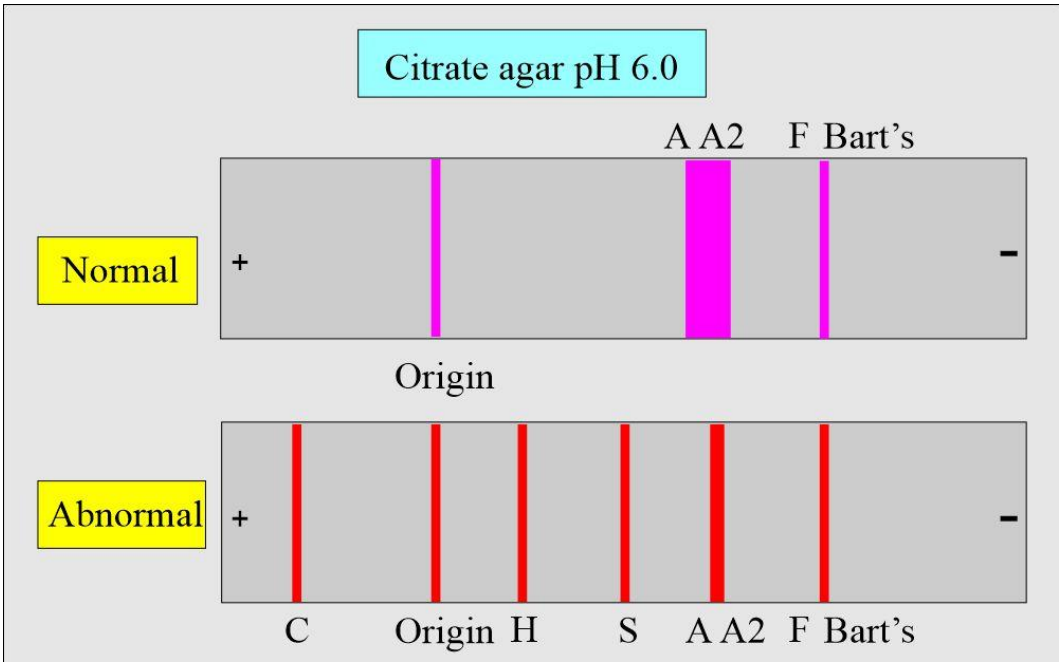

Fig 04: Hemoglobin electrophoresis [Flabpedia :online]

1.1.6 Prevention

The American Society of Obstetricians and Gynecologists has issued a strong recommendation that anybody who is contemplating becoming pregnant be tested to discover whether or not they have thalassemia. The test will determine whether or not the individual has the trait that causes thalassemia [41]. It is recommended very strongly that families who have been identified as carrying a thalassemia trait seek genetic counselling and have genetic testing performed. Cyprus has implemented a screening programme with the intention of reducing the overall number of people who are affected by thalassemia. Since the program's commencement in the 1970s (which also included prenatal screening and abortion), the number of infants born with the condition has decreased to nearly nil from its previous rate of one in every 158 births [42]. In addition, Greece has a screening programme with the purpose of identifying persons who are carriers of the disease [43]. Examining the man's red cell indices is the first stage in Iran's premarital screening procedure. This is done to rule out any potential health risks. If the male has microcytosis, which is defined as having a mean cell haemoglobin of less than 27 pg or a mean red cell volume of less than 80 fl,

then the woman will be evaluated. Microcytosis may also be described as having a mean cell haemoglobin of fewer than 80 fl. At the time that they are in the microcytic state, it is feasible to evaluate both of their haemoglobin A2 concentrations. If both of them have a concentration that is more than 3.5%, which is the diagnostic threshold for the thalassemia trait, then they are sent to the health post that has been established locally for the purpose of receiving genetic counselling [44]. Large-scale public awareness campaigns are now being organised in India [45] by both government and non-government organisations, with the goal of persuading individuals to participate in voluntary premarital tests. This is being done with the intention of reducing the number of marriages that include two carriers of the disease.

1.1.7 Management

Mild thalassemia: Those who have thalassemia characteristics do not need further medical treatment or follow-up care after they have received their first diagnosis of mild thalassemia. [46] Individuals who have the -thalassemia trait need to be made aware that their disease has the potential to be misinterpreted as the more prevalent iron-deficiency anaemia. People need to steer clear of taking iron supplements on a regular basis, although iron deficiency may arise during pregnancy or as a result of persistent bleeding [47]. It is recommended that people who have genetic abnormalities seek counselling, particularly in cases when members of the family are at increased risk for a severe form of an illness that may be avoided [48].

Anemia

Those with severe forms of the disease are required to get medical therapy. The first step in effectively extending one's life is to begin a schedule of blood transfusions [46].

Treatment with growth hormone

There is some evidence to suggest that treating children with thalassemia with growth hormone replacement treatment may assist accelerate the pace at which these children reach their full height potential [49].

An excess of iron

Iron overload is a possible side effect of receiving many blood transfusions. Chelation treatment, which may be combined with the drugs deferoxamine, deferiprone, or deferasirox, may be used to

treat the iron excess that is associated with thalassemia. [50] Patients diagnosed with thalassemia major now have a greater life expectancy as a direct consequence of the therapies available. [50] Since deferoxamine can only be successful when administered through daily injection, its usage over the long term is complicated. On the other hand, it does not cost much and is risk-free. The principal skin responses that might occur near the injection site, as well as hearing loss, are considered adverse effects [50]. Both deferirox and deferiprone are oral drugs that may cause a variety of unpleasant side effects, the most frequent of which are nausea, vomiting, and diarrhoea. While deferiprone appears to be the most effective agent when the heart is involved, deferrasirox is not effective for all patients and may not be appropriate for those who have significant cardiac issues related to iron overload. However, deferrasirox is effective for patients who do not have significant cardiac issues. In addition to this, the expense of deferasirox is a considerable factor [50]. Zinc supplementation has not been shown to be beneficial for those with thalassemia in randomised controlled trials [51].

The transfer of bone marrow

When performed on young patients with an HLA-matched donor, bone marrow transplantation has the potential to give the prospect of a cure [52]. The percentage of successful attempts has increased to the 80–90% level [52]. There is an approximate 3% chance of death as a result of the surgery [53]. There have been no patients with beta-thalassemia who are reliant on blood transfusions who have participated in any randomised controlled studies that have assessed the safety and effectiveness of bone-marrow transplantation from a non-identical donor [54]. One significant risk associated with bone marrow transplantation is the development of graft-versus-host diseases (GvHD). More study is required to determine whether or not mesenchymal stromal cells can be used as a preventative measure or therapeutic therapy for GvHD [55]. Bone marrow transplantation from a haploidentical mother to her kid, often known as a mismatched donor, may be tried in cases when the patient does not have access to an HLA-matched, suitable donor. The percentage of thalassemia-free survival was 70%, the rate of rejection was 23%, and the rate of death was 7% in a study of 31 persons. The most promising outcomes often occur in very young children and adolescents [56].

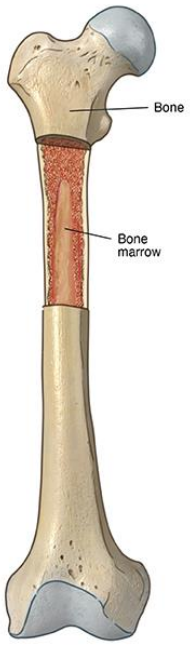

Fig 05: Bone marrow [HOPKINSmedicine :online]

1.2 Gene therapy

Gene therapy is a subfield of medicine that focuses on the genetic modification of cells in order to produce a therapeutic effect [57] or the treatment of disease by repairing or reconstructing genetic material that has been damaged in some way. This can be done in order to treat genetic damage that has been caused by disease. These types of treatments have the potential to be used to treat a broad variety of medical issues. [58] The first attempt to edit human DNA was made by Martin Cline in the year 1980, but it wasn't until May 1989 that the National Institutes of Health granted their clearance for the first successful nuclear gene transfer in humans. Martin Cline was the first person to make an attempt to modify human DNA [59]. French Anderson achieved both the first therapeutic use of gene transfer and the first direct insertion of human DNA into the nuclear genome during a clinical trial that started in September of 1990. The experiment was carried out in a hospital setting. The completion of each of these accomplishments occurred at the same time. It is considered to have the potential to heal or cure a significant number of hereditary conditions over the course of time.Between the years 1989 and December 2018, about 2,900 clinical studies were conducted, with more than half of them falling into the phase I category. These trials were conducted to investigate new treatments for diseases [60].The gene therapy known as Gendicine

was the first of its type to get regulatory clearance in 2003. This accomplishment made it the first of its kind. Glybera (2012), Strimvelis (2016), Kymriah (2017), Luxturna (2017), Onpattro (2018), Zolgensma (2019), Abecma (2021), Adstiladrin, Roctavian, and Hemgenix are just some of the other gene therapy medications that have now been given the go-ahead for use in humans. Others include: Glybera (2012), Strimvelis (2016), Kymriah (all 2022). Adeno-associated viruses (AAVs) and lentiviruses are used in the majority of these approaches to carry out gene insertions, either in vivo or ex vivo, respectively. These methods may be divided into two categories: in vivo and ex vivo. AAVs are distinguished from other viral vectors by the fact that they are able to stabilise the viral capsid, have a lower immunogenicity, transduce cells that are dividing as well as cells that are not dividing, have the potential to integrate site-specifically, and achieve long-term expression in in-vivo treatments. Additionally, AAVs are able to transduce cells that are not dividing as well as cells that are [61]. Approaches that use ASOs and siRNAs, such as those carried out by Alnylam and Ionis Pharmaceuticals, call for the use of non-viral delivery vehicles and make use of alternate routes for trafficking to liver cells by means of GalNAc transporters. This is because these approaches require the use of non-viral delivery vehicles and because they use alternative routes for trafficking to liver cells.It is essential to keep in mind that not every kind of medical procedure that modifies a patient's genetic make-up may be categorised as gene therapy. This is an essential point to keep in mind. It has been revealed that some people may acquire foreign DNA as a consequence of undergoing bone marrow transplantation and organ transplantation in general. This discovery was made possible by the fact that bone marrow transplantation was discovered [62].

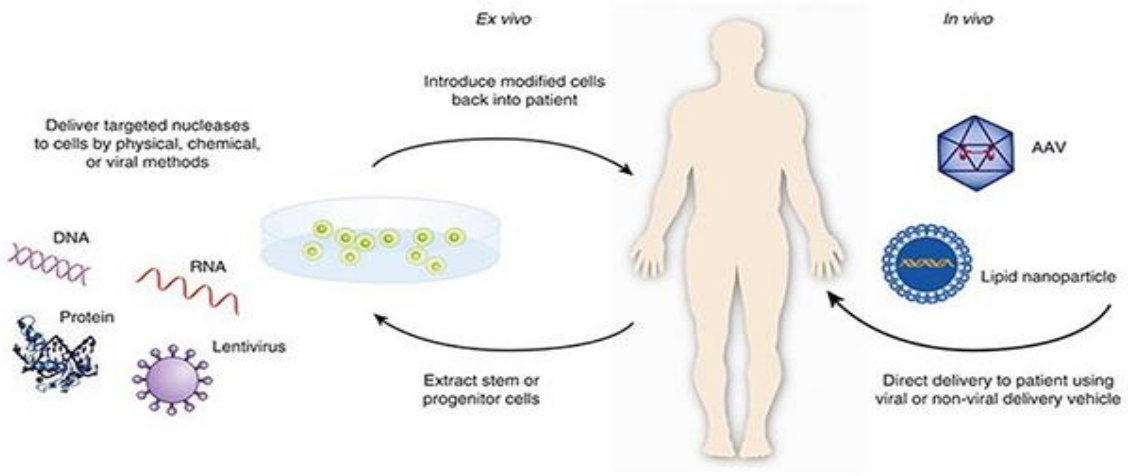

Fig 06: Gene therapy [FDA :online]

1.3. Gene therapy based on cells for thalassemia

The inability to produce sufficient amounts of haemoglobin is a hallmark of the thalassemia syndrome, a group of inherited blood disorders. Alpha thalassemia and beta thalassemia are the two basic subtypes of thalassemia, which may be distinguished from one another. The formation of beta thalassemia is caused by mutations in the HBB gene, which gives the instructions for producing beta-globin, a component of haemoglobin. [63] These mutations may only occur in certain individuals.There is hope that gene therapy may one day be able to cure beta thalassemia, a hereditary condition that affects red blood cells. Cells are extracted from the patient and then undergo the process of genetic modification in a laboratory in order to correct the genetic mutation that is the root cause of the illness. Gene therapy carried out in cells is referred to as cell-based gene therapy. When the cells have been altered, they are reintroduced into the body of the thalassemia patient so that they may produce normal beta-globin and relieve some of the symptoms that are associated with the disease in the thalassemia patient.In cell-based gene therapy intended for the treatment of beta thalassemia, one of the potential therapeutic modalities is the application of hematopoietic stem cells (HSCs) [64]. These are the cells that have the ability to differentiate into a wide variety of blood cells, one of which is the red blood cell, which is responsible for transporting haemoglobin throughout the body. HSCs are harvested from the patient and then subjected to genetic editing with the use of a viral vector that carries an unaltered copy of the HBB gene. After that, the HSCs that have been altered are reintroduced into the patient. When the cells have been altered, they are re-infused into the patient in the hopes that they would promote the creation of healthy red blood cells as well as an improvement in the symptoms that are experienced by the thalassemia patient. Another kind of cell-based gene therapy that may be used in the treatment of beta thalassemia is known as the utilisation of erythroid progenitor cells (EPCs). These cells are farther along in the process of becoming red blood cells than HSCs are, and as a consequence, they are more committed to completing this process and becoming red blood cells. These cells are farther down the process than HSCs, which are not yet fully differentiated. EPCs may be harvested from the patient's bone marrow or from induced pluripotent stem cells (iPSCs), which are produced from the patient's own cells. EPCs are more likely to be found in the bone marrow. EPCs are used in the treatment of a wide range of illnesses, one of which being cancer. After then, the EPCs go through the process of genetic alteration so that they can produce beta-globin that is in good health. Following this, they are reintroduced into the body of the patient,

who is then provided with the capacity to produce healthy red blood cells.The use of cell-based gene therapy as a treatment for thalassemia is still in the research and development stage, and it has not yet been given the go-ahead for widespread clinical usage. Currently, this treatment is only available to a limited number of patients. Despite this, early study has showed some hopeful results, including improved levels of haemoglobin and a decreased need for blood transfusions in individuals who were given medication [65].

1.4. Gene therapy performed on living cells (zynteglo)

Beta-thalassemia is a kind of hereditary haemoglobin condition that makes it difficult for the body to create its own haemoglobin. This illness may be passed down from parent to child. The protein known as haemoglobin may be found inside of red blood cells. This protein is essential for the transport of oxygen throughout the body [66]. Blood contains a protein called haemoglobin. Zynteglo is a kind of gene therapy that makes use of a treatment that is based on cells. It is used to treat beta-thalassemia, which is a blood disorder. The treatment includes introducing a functional copy of the beta-globin gene to the patient's own hematopoietic stem cells via the use of a viral vector. These cells come from the patient.

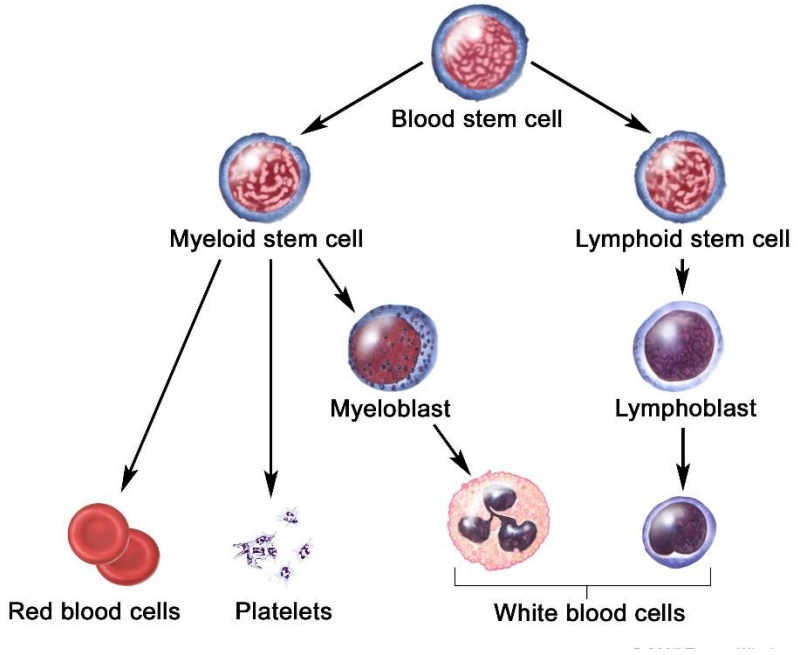

Fig 07: Hematopoietic stem cells [cancer.gov:online]

These cells originate from the individual being treated (HSCs). Following that, the cells are reintroduced into the patient's body through a procedure known as a transplant [67].When the HSCs have been altered, they are reintroduced into the patient so that they can maintain the production of healthy red blood cells and reduce the effects of beta-thalassemia. The European Medicines Agency (EMA) granted approval for the use of Zynteglo in 2019 for the treatment of patients with transfusion-dependent beta-thalassemia who are at least 12 years old and do not have a donor who is suitable for a stem cell transplant. These patients are eligible for Zynteglo only if they do not have a donor who is suitable for a stem cell transplant. Some patients do not meet the criteria to get a transplant of stem cells. But, as of this point in time, the Food and Drug Administration in the United States has not yet given its approval for it (FDA). Patients who took part in the study reported an improvement in their quality of life and a reduction in the frequency of their need for blood transfusions as a result of Zynteglo's use. These are both positive findings from the clinical trials that have been carried out, and they indicate that Zynteglo is effective. Since it is such a difficult, time-consuming, and costly therapy that necessitates the use of specialised equipment and people, it is presently inaccessible to a substantial portion of the population around the globe [68].

Chapter-2
Goal of the Study

2. Goal of this studies

My aim of this study was,

- To overview the current situation of thalassemia diseases.
- To find out the current treatment of thalassemia.
- To find the gene therapy for thalassemia treatment.
- To open a new area of higher studies.

Chapter-3
Methodology

3.Methodology

3.1. Introduction:

A literature review leads the examination. Around 71 papers are reviewed for this study.

3.2. Research Design:

This exploration was planned through google scholar, PubMed and many other websites to find literature.

3.3. Method of Data Analysis:

After compiling a large amount of information, it was examined for accuracy and coherence within itself to rule out the possibility of missing or conflicting data, both of which were afterward thrown out. The Microsoft Dominant Refreshed Version was used in the information inquiry that was carried out. All of the information gathered is from 1989 all the way until 2022.

3.4. Ethical Considerations

Before beginning the process of information collection, informed verbal consent was obtained from those participating in the inquiry. A veil of secrecy was maintained over the identities of those who participated in the research, and participants in the studies were informed that they were free to withdraw from the program at any point throughout the process of data collection. The inquiry garnered the backing of the Department of Pharmacy.

Chapter-4
Result & Discussion

4. Result & Discussion

4.1. Bone marrow transplantation for thalassemia

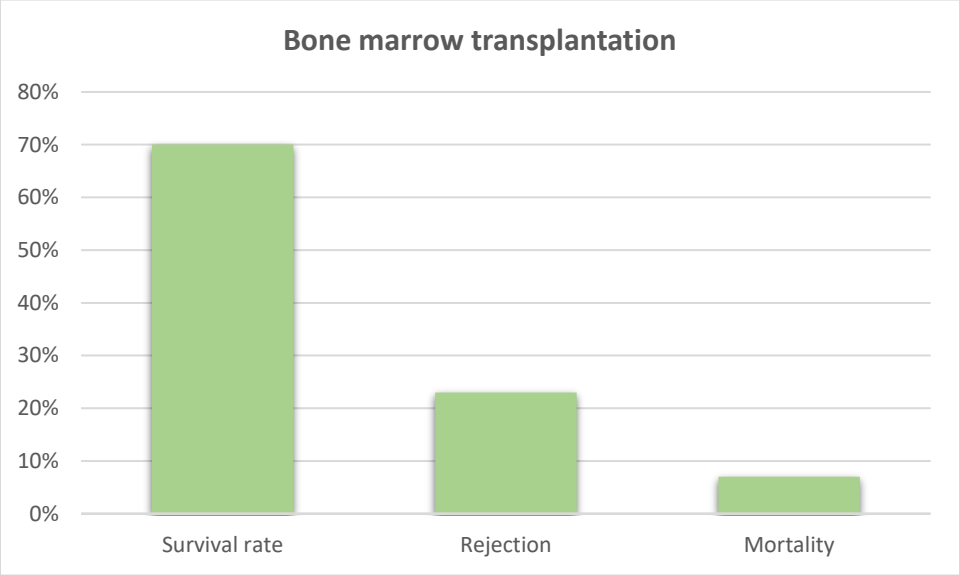

Fig 08: Bone marrow transplantation for thalassemia

According to the review study, young patients who have an HLA-matched donor may be able to get a cure via bone marrow transplantation. Bone marrow transplantation from a child of a haploidentical mother (a mismatched donor) may be tried if the patient lacks an HLA-matched suitable donor. In a trial including 31 individuals, rejection was 23%, mortality was 7%, and thalassemia-free survival was 70%. Younger individuals often have the best outcomes [69][70].

4.2. Number of deaths for thalassemia

Year	Number of deaths
1990	36000
2013	25000

Table 01: Number of deaths for thalassemia

The beta type of thalassemia is reportedly more common among Mediterranean peoples, and its original name derives from this geographic relationship, claims the research article. In comparison to 36,000 fatalities in 1990, thalassemia caused 25,000 deaths in 2013.

Greece, Turkey's coastal districts (especially those along the Aegean and Mediterranean Seas, including Antalya, Adana, and Mersin), southern Spain, and sections of Italy, notably southern Italy, have the highest rates of the illness in Europe. Major Mediterranean Islands including Sicily, Sardinia, Malta, Corsica, Cyprus, and Crete, with the exception of the Balearics, are severely impacted [71][72].

4.3. Hemoglobin type and their percentage in Beta thalassemia

Thalassemia condition	**HbA**	**HbA2**	**HbF**
Unaffected	97%	2%	1%
B-thalassemia major	0%	Remainder	Over 95%
B-thalassemia Intermediate	Remainder	Over 2%	30-90%
B-thalassemia minor	Remainder	2-8%	Up to 30%

Table 2: Hemoglobin type and their percentage in Beta thalassemia

The review study states that the forms of hemoglobin—HbA (adult hemoglobin), HbA2 (adult hemoglobin type 2), and HbF—determine the beta form of thalassemia (Fetal hemoglobin) [73].

4.4. Blood transfusion complications in thalassemia patients

Infections	Virus Bacteria Parasite
Hemolytic Reactions	Acute hemolytic reactions Delayed hemolytic reactions Autoimmune hemolytic reactions
Non-Hemolytic Reactions	Allergic and anaphylactic reactions Febrile non-hemolytic reaction Transfusion-related acute lung injury (TRALI) Circulatory overload

Table 03: Blood transfusion complications in thalassemia patients

According to this research, complications for blood transfusion in thalassemia patients might occasionally be noticed, such as anaphylaxis or allergic shock, which can also lead to infection with viruses or bacteria. Protozoan [74].

Chapter-5
Conclusion

5.Conclusion

Thalassemia is a group of inherited blood disorders that affect the production of hemoglobin, a protein found in red blood cells that carries oxygen. Zynteglo is a type of gene therapy that uses a patient's own stem cells to treat transfusion-dependent beta-thalassemia. It involves modifying the patient's stem cells outside the body using a viral vector to introduce a functional copy of the beta-globin gene, which produces the missing hemoglobin. The goal of the therapy is to reduce or eliminate the need for transfusions, which can be a significant burden for people with thalassemia. However, gene therapy is a relatively new field and long-term safety and efficacy data are still being collected.

Chapter-6
References

6. References