lineages (including the erythroid), are collected from the patient in a hospital setting, processed in the laboratory with a vector carrying the correct gene, and infused back to the patient in the hospital after depleting the thalassaemic bone marrow once again in the hospital setting. (More info at https://www.zynteglo.eu/)

WHAT IS GENE-BASED CURE? WHAT ARE THE STEPS INVOLVED WHEN USING THE RECENTLY APPROVED PRODUCT ZYNTEGLO ®?

Steps 1 and 4 outlined below happen in the hospital, steps 2 and 3 in a specialized laboratory.

1) Medical specialists, (haematologists/transplanters) collect cells from the peripheral blood of the patient. In the latter case, the patients receive specific drugs to help them increase the number of the appropriate *primitive haemopoetic CD34+ cells* that come out of the bone marrow into the peripheral blood i.e *to help induce their migration from their natural "home" which is the bone marrow to the peripheral blood of the patient. This process is known as* ***stem cell mobilization***.
2) The target cells (CD34+ cells) are then processed/cultured in the laboratory in the presence of special factors (growth factors) to help them self-renew, then grow and expand. It is in these cells that the "correct", "functioning" β-globin gene will be inserted. This is achieved with the help of a "carrier" of the gene of choice or *"vector"*. This is a structure that needs to be very small and possess the capacity to enter the target cells transferring the "correct" β-globin gene. In this approach, using the Zynteglo® protocol, the vector used is a virus belonging to the family called *Lentiviruses*. These viruses, which under normal conditions are highly infectious and can cause serious disease in humans, undergo modifications in the laboratory, prior to their use, that is a process through which *the infectious structures are destroyed/removed*. The viruses thus become safe for use in humans while at the same time they maintain their ability to enter the cells' nucleus and integrate correct beta globin gene into the DNA of the human cells (CD34+ cells). Once the viral vector delivers the genetic information into the cell, it cannot assemble and is now inactivated. The progeny from the human CD34+ cells with integrated correct beta globin DNA will inherit this gene, *maintaining the therapeutic benefit life long.*
3) The transfer of functional beta globin gene is achieved when the vector (i.e. the virus) containing the β-globin gene is co-cultured with the collected/harvested CD34+. This process is known as *transduction* and describes the transfer of the "correct" genetic information i.e. the β-globin gene into the CD34+ cells.
4) *The CD34+ cells now containing the "correct" or normal β-globin gene are known as the "product"*. When all safety and quality controls are completed, this product is *reinfused* into the blood circulation after the patient has received a special treatment called *conditioning*. This is a kind of chemotherapy that oncology patients receive and is necessary in order to suppress the bone marrow of the patient providing space within it, to receive the "product". Once the "product" is REINFUSED into the patient it will find its place in the bone marrow - a process referred to as *engraftment*.

   In this way the haemotopoietic system expresses functional hemoglobin from the stem cells with added beta globin gene (Fig. 8[3])

Besides the conditionally approved therapeutic indications of Zynteglo[4], patients need to meet some inclusion criteria in order to safely undergo this treatment: *Generally, apart from certain genetic characteristics,*

(i) They need to be in good clinical condition;
(ii) Be adequately chelated and transfused;
(iii) Have acceptable acceptable cardiac, renal and hepatic function and;
(iv) They must be over 12 years of age and able, in terms of safety, to receive the drugs for
   a. stem cell mobilization, and
   b. conditioning before the gene product infusion.

Every patient who is interested to receive this treatment must have a very thorough discussion, on an individual basis, with both his/her treating and transplant physicians regarding his/her eligibility.

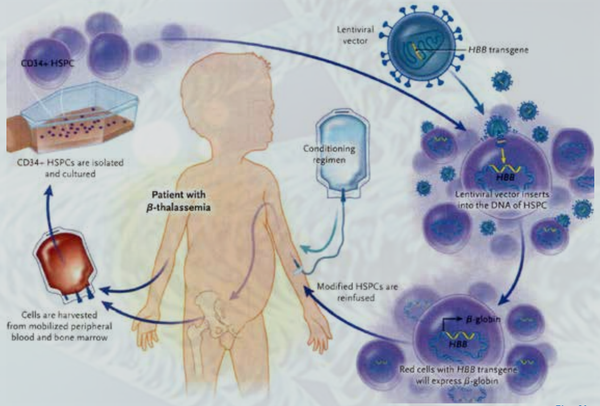


Fig. 8[3]

[3] From the New England Journal of Medicine, 2019; 381:455-464, DOI: 10.1056/ NEJMra1706910
[4] Zynteglo® has been given 'conditional authorisation'. This means that there is more evidence to come about the medicine, which the company is required to provide. Every year, the European Medicines Agency will review any new information that becomes available and this overview will be updated as necessary.' More details about conditional approval process can be found here: ema.europa.eu/en/human-regulatory/marketing-authorisation/conditional-marketing-authorisation'

With permission requested, taken from the New England Journal of Medicine, 2019; 381:455-464, DOI: 10.1056/ NEJMra1706910

Glossary:

Haemopoiesis - the process of producing blood cells

Haemopoietic cells - cells which are undergoing differentiation steps (maturation) to produce mature blood cells.

Mobilisation - a process in which certain drugs are used to cause the movement of primitive hematopoietic cells (stem cells) from the bone marrow into the blood. CD34 positive cells (CD34+) – They are early stem (progenitor) cells which go on to form mature blood cells. They are 'mobilised' and collected by a process called leukapheresis, which allows large numbers to be collected from peripheral blood.

HSPCs - sho rt for haemopoietic stem, progenitor, cells, i.e. early stem cells capable of producing various kinds of blood cells.

Transduction - the transfer of genetic material (DNA) by a viral vector into the cultured cells of a patient outside the body.

Conditioning - this is the process of preparing the patient's bone marrow to receive the genetically modified stem cells. It is achieved by destroying or minimising the patient's own haemopoietic tissue by chemotherapy (in this case) to make room for the new, treated, stem cells.

Engraftment - finding the correct place in the bone marrow which would lead to the growth of haemopoietic stem and progenitor cells.

THALIA

This Educational Brochure for the Patient entitled "Explaining Gene Therapy in Thalassaemia" received funding under an operating grant from the European Union's Health Programme (2014-2020).

Co-funded by
the Health Programme
of the European Union

© Thalassaemia International Federation 2020
All rights reserved.

Thalassaemia International Federation
31 Ifigenias Street, 3rd Floor, 2007 Strovolos, Nicosia Cyprus
P.O. Box 28807, 2083 Strovolos, Nicosia Cyprus
Tel: +357 22 319 129 • Fax: +357 22 314 552
E-mail: thalassaemia@cytanet.com.cy
Website: http://thalassaemia.org.cy

Thalassaemia International Federation (TIF) can help you resolve many of your queries and questions through its International Medical and Scientific Advisory Board and educational programme.

- Please visit TIF's website (http://thalassaemia.org.cy)
- Request additional educational material on Gene Therapy (Gene Therapy in β-thalassaemia booklet) which will be sent to you free of charge.
- An update on all clinical trials related to Thalassaemia and other Hb disorders.
- Contact TIF's helpline at +357 22 319 129 [operates between 8:00 am to 4:00 pm local time (GMT+2)]

Please complete TIF's questionnaire on the patient's perspective with regards to Gene Therapy. **Your views matter to us and guide our work and activities.**

https://www.surveymonkey.com/r/FK3P223
or **scan the QR code**

Your comments are very welcome

explaining
GENE THERAPY
in thalassaemia

THALASSAEMIA INTERNATIONAL FEDERATION

Thalassaemia is a genetic, hereditary disorder.
Hereditary means that this disorder is passed on from parents to their children and genetic describes the WAY it is passed on through the **GENES**. Different diseases are passed on (or inherited) in different ways.

Thalassaemia is inherited in a way known in biology and genetics as *'Mendelian'* (from the name of the monk/scientist who described it), *autosomal*, because it can affect males and females alike, and *recessive*, because the child needs to inherit two "defective" globin genes: one from the father and one from the mother, in order to develop the severe form of the disorder, in this case β-thalassaemia major.

WHAT ARE GENES?

They are the biological building blocks of our body. They are made up of a chemical substance known as *D*eoxyribo*N*ucleic *A*cid or *DNA* for short, and which is a thread-like structure with many threads linked together into 23 pairs known as CHROMOSOMES *(Fig. 1)*. 22 of these pairs are the same in both males and females and are called *autosomes*. The 23rd pair is different between males and females exactly because it determines the gender, i.e. whether the individual is male or female.

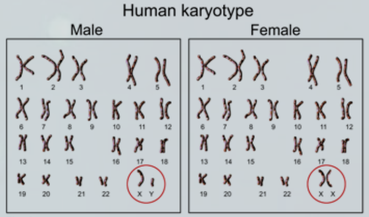


Fig. 1

WHAT DO GENES DO?

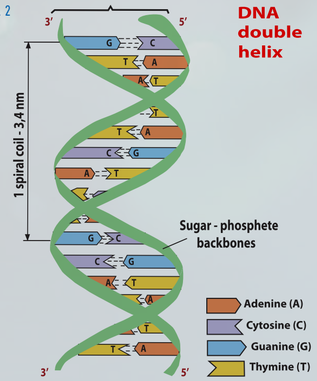
A great number of GENES are needed to carry out the many and complicated biological functions of the human organism. They in fact provide all the biological information needed by an individual organism for controlling life, growth and development. They are, in other words, the unique blueprints of every individual and constitute the biological units of INHERITANCE.

THE DNA CONTENT OF THE GENES – A CLOSER LOOK

The DNA *(Fig. 2)* making up the genes, resembles a ladder with two parallel structures, also referred to as double helix, that supports a series of rungs, each one composed of two chemical structures, called bases.

These are *paired* in a very restricted way: *A (Adenine) can only pair with T (Thymine) and C (Cytosine) can only pair with G (Guanine)*. *(Fig. 3)*

Fig. 2



The order (sequence) in which these bases are laid along this "ladder" constitutes in fact the individual's genetic code. These sequences taken together as a whole constitute what is commonly known as the "genome" of the individual. This contains thousands of genes.

DNA – GENES – CHROMOSOMES FOR HAEMOGLOBIN

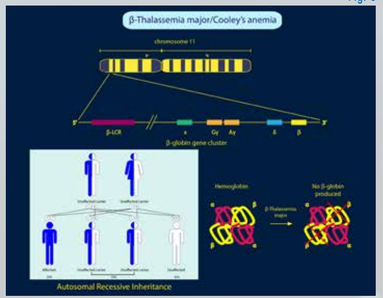
Different genes control the production of various essential proteins for the normal growth and functioning of the human body.

GENES in chromosomes *16* and *11* (out of the 23 pairs of chromosomes in an individual), for example, are responsible for the production of an essential part (called globin) of a protein known as *haemoglobin*. This is an important constituent of blood and more specifically of the Red Blood Cells (RBCs). *(Fig. 3)*

The main function of haemoglobin is to transport to all cells, tissues and organs of the human body *oxygen*, an essential *nutrient* for their normal function.

Three hundred (300) million molecules of haemoglobin are contained in EACH Red Blood Cell.

Fig. 3



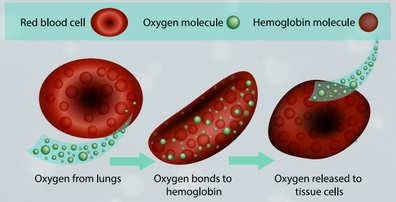
HAEMOGLOBIN STRUCTURE

A molecule of Haemoglobin has two parts:

The first part is globin and consists of four (4) protein structures, known as *chains*, arranged in matching pairs. The normal adult globin is made up of *equal amounts* of α (alpha) and β (beta)-chains matched as $\alpha_2\beta_2$ and their production is regulated by genes on chromosome 16, controlling the production of α-chains, and chromosome 11, controlling the production of β-chains. *(Fig. 3)*

The second structure of haemoglobin *(Fig.4)* is the *haem - iron* part. This is a ring structure, situated in the centre of each of the four chain bundles, which contains an *iron* molecule (*Fig. 5)*. IRON is an element which is responsible for the red colour of the RBCs and by extension for the colour of human blood. However, very importantly, iron has the property to *bind* and *release oxygen* easily, making it the perfect vehicle for the RBCs to perform their role thus transferring the oxygen across the body. 3g[1] out of 4g (75%) of iron in the body is used to synthesize haemoglobin.

Fig. 4



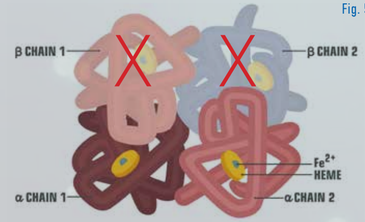
WHERE ARE THE RBCs AND OTHER BLOOD CELLS PRODUCED?

The *bone marrow* is the site of production of blood cells, including the progenitor cells of the RBCs. Bone marrow is a tissue formed in the central cavity of the bones e.g. in the skull, spine, ribs and pelvis in adults and in additional bones in children.

What is happening in β-thalassaemia major?

In β-thalassaemia there is a *defect* or a *mutation* (in biology/genetics) in the DNA of the genes *(Fig.5)* of chromosome 11, controlling the production of the β chains of the globin molecule. Other genes on chromosome 16, as mentioned above, control the production of α-chains of the globin molecule. As a result of the mutation in chromosome 11, the genes cannot perform their normal function. Consequently, very little or no β-chains are produced and the production of "normal"/"functional" haemoglobin, which requires equal amounts of α-chains and β-chains, is severely affected. This chain imbalance is the major cause of the pathophysiology and all the severe clinical outcomes of β-thalassaemia major.

Fig. 5



[1] g = gram

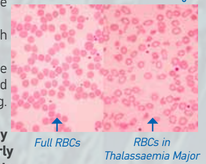
WHEN DOES THIS HAPPEN?

This happens when a child inherits from each of its parents one mutated, non-functional β-globin gene and the major clinical outcomes include:

(i) Severe anaemia (since RBCs have an inadequate amount of normal adult Hb - they do have some foetal haemoglobin - Hb-F) *(Fig. 6)*;
(ii) Skull and other bone deformities;
(iii) Restricted normal growth and physical activity and;
(iv) And many and multiple other organ-related complications, e.g. hepatosplenomegaly.

In the absence of any medical intervention, early death prior to patients reaching the second decade of their lives occurs. *(Fig.7)*

Fig. 6



HOW IS B-THALASSAEMIA MANAGED?

Through the decades, particularly after the 1960s, considerable work by the medical and scientific communities was focused on identifying those elements that would support the survival of patients with this disorder, thus leading by the 1990s, to the development of effective drugs and management schemes. This did not only allow patients to survive, but very importantly, to live well, have a decent quality of life and, to a great extent achieve almost full social integration (at least in those countries which prioritized the disorder on their national health agendas). The essential medical interventions include: **(i) Lifelong, regular (at least monthly) blood transfusions with filtered RBCs appropriately stored, fresh and fully matched AND (ii) iron chelation to remove the iron deposited in the vital organs of the body mainly resulting from the breakdown of the transfused RBCs when they complete their normal life cycle in the circulation.**

In addition to iron chelation treatment *multidisciplinary care* is essential to achieve good survival rates and a good quality of life (according to TIF's International guidelines[2]).

[2] Guidelines for the Management of Transfusion Dependent Thalassaemia, 3rd Edition (2014)

Summary of what eventually happens in untreated thalassaemia major

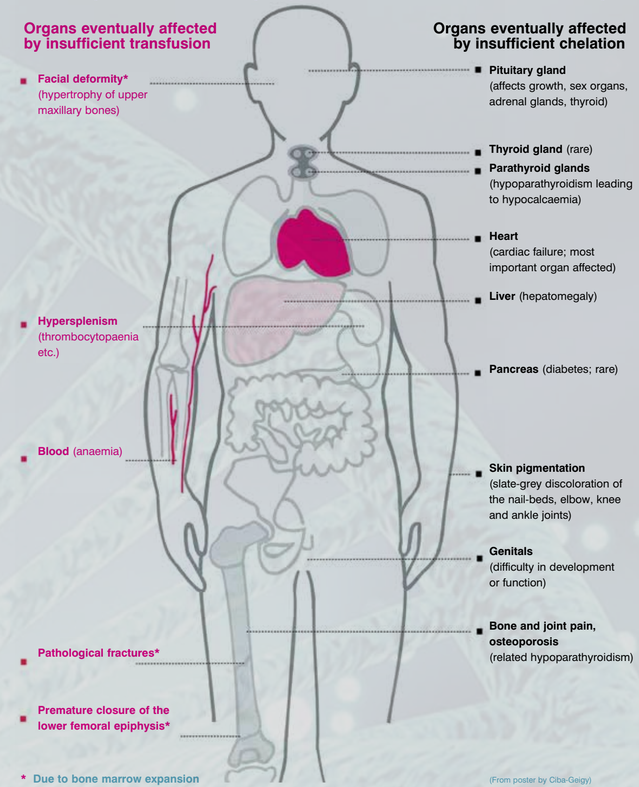


Fig. 7

GENE-BASED METHODS FOR THE TOTAL CURE OF THALASSAEMIA

For many decades, research activity focused on identifying ways to eventually CURE this disorder. Haematopoietic stem cell transplantation (HSCT), more commonly known as Bone Marrow Transplantation (BMT), has contributed significantly to the realisation of this vision. BMT, meaning the infusion of healthy hematopoietic cells collected from a matched donor after eliminating the patient's defective bone marrow cells, offered good to excellent outcomes throughout the years. Particularly true for those at younger ages and with an HLA-identical sibling donor, a prerequisite which sadly is only possible in a restricted percentage of patients not exceeding 20-30%. Research work for more improvements in this avenue with the use of matched non-sibling (unrelated, volunteer) and non-identical related donors is ongoing: Nonetheless, success similar to the identical sibling match has not yet been established with the exception of very few expert BMT centres.

For decades, research in academia and industry has also been focusing on gene-based solutions for the total cure of thalassaemia. The aim was, and still is, to replace, correct the defective globin GENE, or add a functional copy of the beta globin gene in the affected individuals. While a number of different approaches are still in progress, a gene addition approach has reached success and has recently received European Medicines Agency's (EMA) approval for its "product", referred to as *Zynteglo®*. This is a "personalized" treatment in which the most *primitive haematopoietic cells, CD34+ cells*, which give rise to all blood cell