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Selecting β-thalassemia Patients for Gene Therapy: A Decision-making Algorithm

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Network Hematological Diseases (ERN EuroBloodNet)

INTRODUCTION

At this point in time, when we can see the emergence of 'the age of gene therapy' and recent preliminary results on gene editing are promising, it is essential to establish the optimal patient setting in which gene therapy can be applied, or better, to define the setting that represents the most suitable indication for gene therapy, identify the patients who should have clinical priority for access to the procedure, and set out requirements and recommendations for the identification of qualified treatment centers for gene therapy.

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Angelucci E. Hematology Am Soc Hematol Educ Program 2010, vol 2010: 465-462

INTRODUCTION

- the expectation of patients is high
- the need to align the specialized centers in Pre-Selecting the patients to refer to the Transplant Centers

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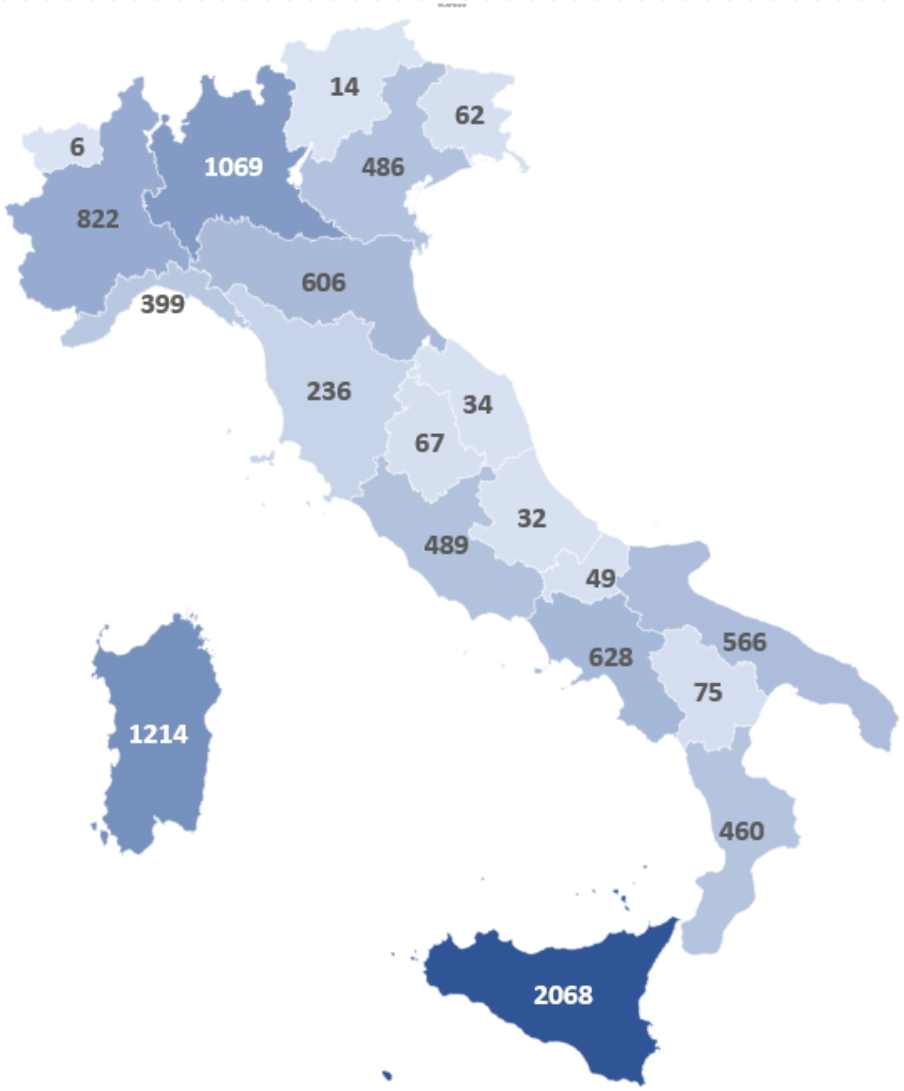
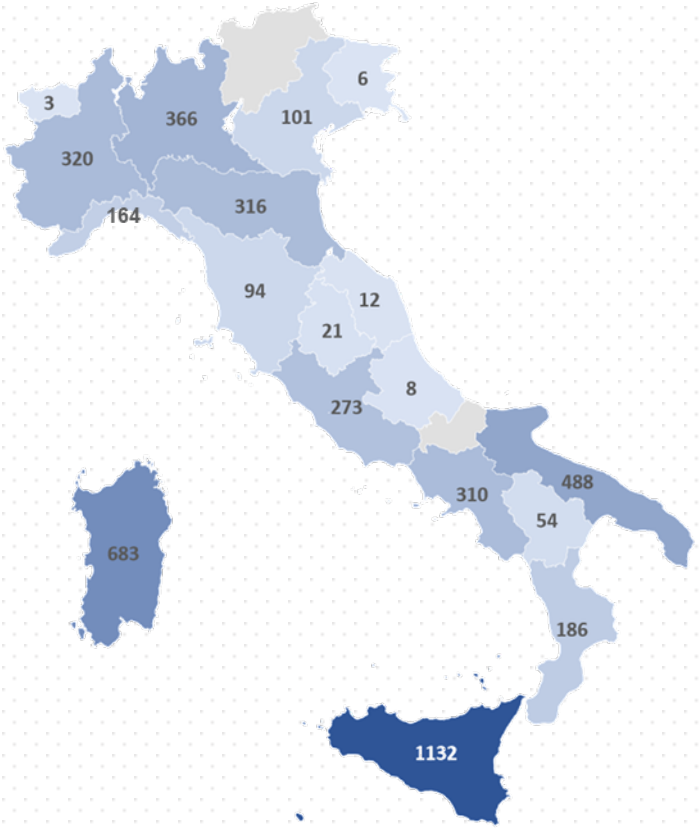
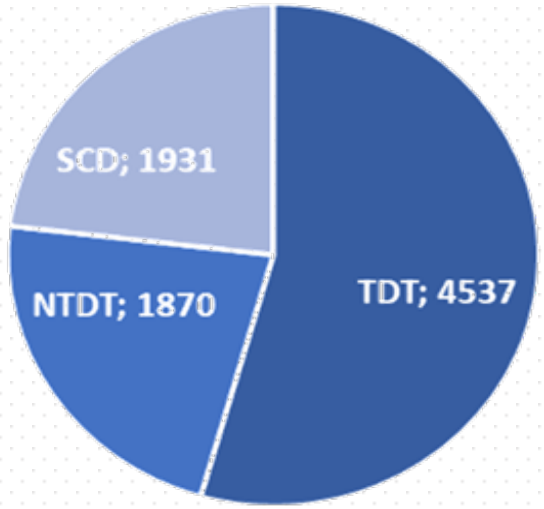
Regional Distribution of Patients with Hemoglobinopaties in Italy



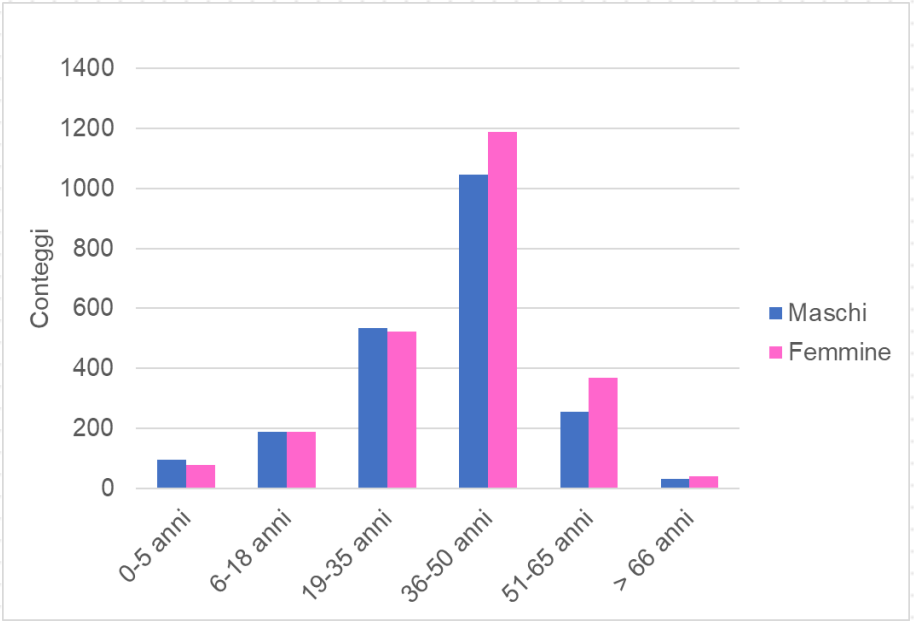
Regions	TDT	NTDT	SCD	Total
Abruzzo	8	12	12	**32**
Basilicata	72	0	3	**75**
Calabria	317	60	83	**460**
Campania	310	229	89	**628**
Emilia-Romagna	329	47	230	**606**
Friuli-Venezia-Giulia	9	3	50	**62**
Lazio	273	68	148	**489**
Liguria	164	130	105	**399**
Lombardia	381	293	395	**1069**
Marche	12	1	21	**34**
Molise	17	21	11	**49**
Piemonte	335	214	273	**822**
Puglia	488	64	14	**566**
Sardegna	827	372	15	**1214**
Sicilia	1340	381	347	**2068**
Toscana	94	17	125	**236**
Trentino Alto Adige	4	1	9	**14**
Umbria	21	14	32	**67**
Valle d'Aosta	3	0	3	**6**
Veneto	137	34	315	**486**
Total	**5141**	**1961**	**2280**	**9382**

TDT patients 4537





Distribuzione per fascia di età e sesso



INTRODUCTION

A consensus document originally developed by an expert panel of the "Italian Society of Thalassemias and Hemoglobinopathies" (SITE), reviewed and adopted by the European Hematology Association (EHA) through the EHA Scientific Working Group on Red Cells and Iron has responded to the need to describe the inclusion and exclusion criteria, and clinical priority to identify which patients with transfusion-dependent beta-thalassemia (β-TDT) could benefit from gene therapy (GT) in the real life

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Guideline Article - Expert opinion

OPEN ACCESS

Selecting β-thalassemia Patients for Gene Therapy: A Decision-making Algorithm

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A consensus document originally developed by an expert panel of the Italian Society of Thalassemias and Hemoglobinopathies (SITE), reviewed and adopted by the European Hematology Association (EHA) through the EHA Scientific Working Group on Red Cells and Iron

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This document is an initiative of the Società Italiana Talassemie ed Emoglobinopatie (Italian Society of Thalassemias and Hemoglobinopathies, SITE) who also financed the project.

Note for users

This document is the full version of the SITE Experts' Opinion and can be downloaded from the SITE website at www.site-italia.org

The members of the panel have signed the Declaration of Conflicts of Interest.

WORKING GROUP

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METHODS

The group would like to emphasize the approach used for evaluating β-TDT patients' access to GT. Until a few decades ago, β-TDT was considered an unfavorable pathology. Today, β-TDT has an open prognosis thanks to the conventional treatment that has transformed it into a chronic disease.

Given this change, it has been decided, in this first phase of access to GT, to give the priority to patients in the best clinical condition who, as shown in the allogenic transplantation setting, are those who will obtain the best results with the least risk.

This cautious approach is due to the limited experience obtained with the registered trials and also to the probable limited availability of the product used, at least initially. The defined priority criteria could vary in the light of new scientific evidence. Given this, the document is to be considered dynamic and up-datable .

INTRODUCTION

The Società Italiana Talassemie ed Emoglobinopatie (Italian Society of Thalassemias and Hemoglobinopathies, SITE) has developed this expert opinion based on multidisciplinary discussions of a panel of experts to provide guidance on the identification and selection of patients with transfusion-dependent beta-thalassemia (β-TDT) who could benefit from gene therapy. The European Hematology Association (EHA), through the EHA Scientific Working Group (SWG) on Red Cells and Iron, has furthered the initiative through an international panel of reviewers and adopted these suggestions. This is the second version, March 2020; translated and edited, August 2020.

Currently, allogeneic transplantation of hematopoietic stem cells is the only curative and most widely used therapy treatment for β-TDT. However, recent trials of gene therapy have reported very promising results in terms of overall survival[1–3] and thalassemia-free survival and are opening a new landscape of treatment. This algorithm for the selection of patients suitable for gene therapy and the supporting notes were formulated by consensus review after an evaluation of currently available scientific evidence using validated criteria. The evidence was interpreted with caution because clinical trial experience of gene therapy is currently limited, a conventional treatment is available for patients with β-TDT and the availability of gene therapy will, at least initially, be quite limited.

Clinical experience of allogeneic transplantation in β-TDT, which began in 1981, immediately showed the importance of patient risk stratification in order to achieve the best results[4–6] (see the Pesaro experience and their classification of patients according to risk). Published data in the literature and the recent analysis of clinical evidence by the European Registry of Hemoglobinopathies of a large number of patients (2011 and 2018 analyses) confirm that young patient age (<14 years) and the availability of a human leukocyte antigen (HLA)-identical family donor are factors that offer the best outcome from allogeneic transplantation[6–10].

Current knowledge of, and experience with, non-conventional treatments, such as allogeneic transplantation and gene therapy, are discussed in order to identify the best available treatment and indication for these patients according to their characteristics.

At this point in time, when we can see the emergence of ‘the age of gene therapy’ and recent preliminary results on gene editing are promising[11], it is essential to establish the optimal patient setting in which gene therapy can be applied, or better, to define the setting that represents the most suitable indication for gene therapy, identify the patients who should have clinical priority for access to the procedure, and set out requirements and recommendations for the identification of qualified treatment centers for gene therapy. When considering changes to the treatment of patients with β-TDT, including gene therapy, it is essential that a detailed consultation is held with the patient and their caregiver/family to discuss all possible risks and potential benefits from the treatment. Discussion of this aspect of care is outside the scope of this expert opinion but remains an important element of patient care.

METHODS

The current expert opinion describes the different clinical problems with practical conclusions on each aspect of the subject, and is summarized by a decision-making algorithm (**Figure 1**) An appendix is also included in the supplementary information, providing details on the requirements and recommendations for the identification of qualified gene therapy treatment centers.

The decision-making algorithm was developed by the SITE group, who defined the project and selected a multidisciplinary group of experts in hemoglobinopathies and/or transplantation to discuss the selection of β-TDT patients for gene therapy and draw up notes on the related clinical problems. The EHA, through the EHA SWG on Red Cells and Iron, has furthered the initiative through an international panel of reviewers and adopted these suggestions. The expert opinion has been prepared to be used by specialists in the centers of the Networks of Hemoglobinopathies.

The published literature (Medline, PubMed, Embase, Cochrane Library) was searched for high-quality evidence to define the best candidates for allogeneic transplantation who should not undergo gene therapy. The keywords used were: Beta-Thalassemia; Bone Marrow Transplantation; Gene Therapy; Hematopoietic Stem Cell (HSC) Transplantation; Hemoglobinopathies; Hepatitis; Iron Overload; Liver Complications; Endocrine Complications.

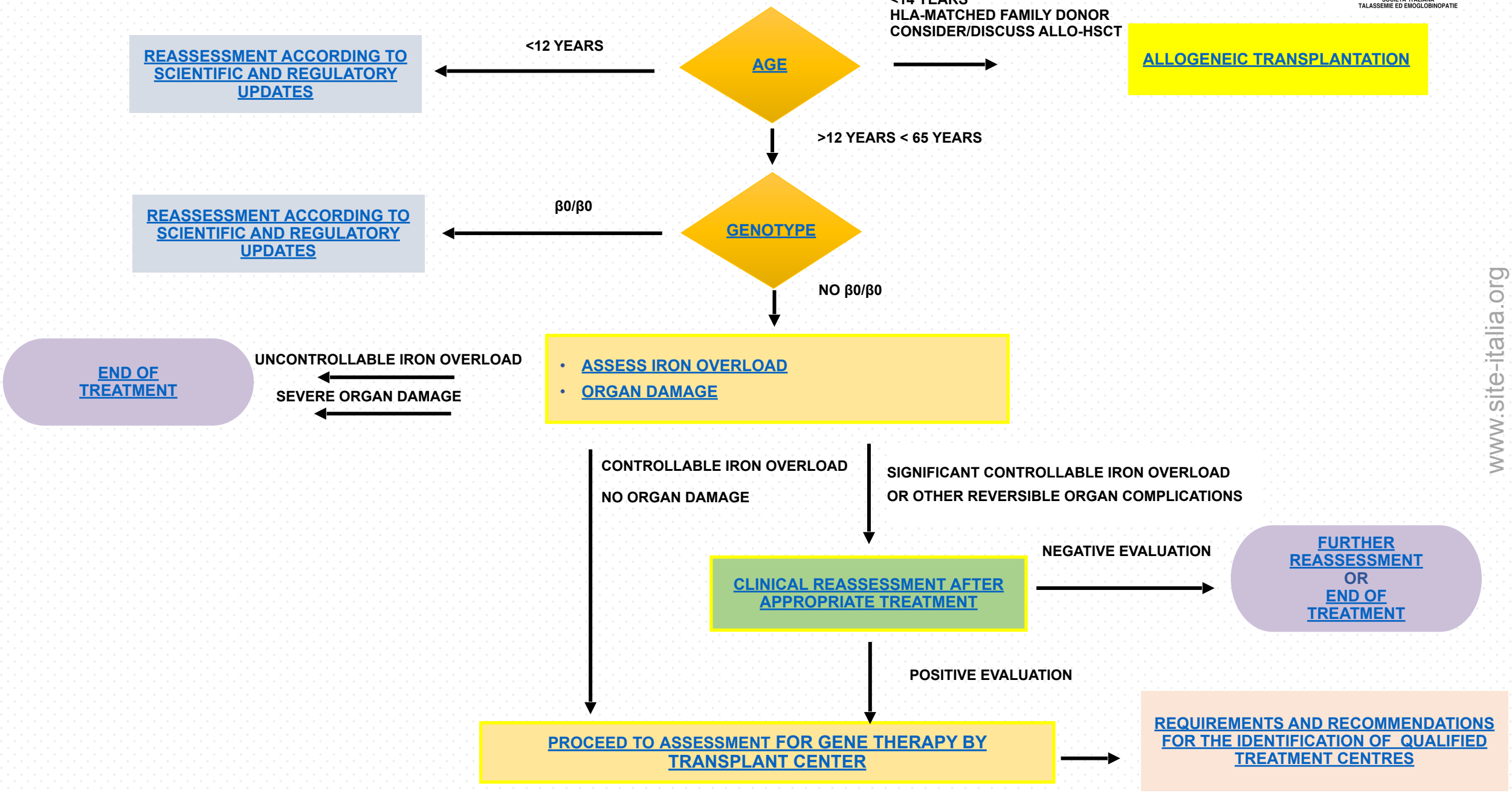
The literature evaluation/scientific evidence was reported and discussed by the SITE expert panel. The final version of the document was then assessed by a pool of external reviewers with modifications made as appropriate and the final version of the expert opinion will be uploaded onto the SITE web site (www.site-italia.org). It is intended that the results of the process will be collected in the clinical electronic database used by the centers (e.g. Webthal®, International Health Repository) to carry out stratification.

The SITE group would like to emphasize the approach used for evaluating β-TDT patients' access to gene therapy. Until a few decades ago, β-TDT was considered an unfavorable pathology. Today, however, β-TDT has an open prognosis thanks to advances in conventional treatment that have transformed it into a chronic disease. It was therefore decided, in this first phase of access to gene therapy, to give priority to patients in the best clinical condition who, as shown in the allogeneic transplantation setting, are those who are likely to obtain the most clinical benefit with the least risk. As mentioned previously this cautious approach is due to the limited clinical experience obtained during clinical trials and due to the probable limited availability of gene therapy, at least initially. The patient priority criteria for access to gene therapy defined in this algorithm and supporting notes could vary as new scientific evidence emerges and therefore the expert opinion will be reviewed regularly and updated when new clinical data are published and/or any changes are made to the European Medicines Agency (EMA) license for gene therapy.

Suitable patients for gene therapy:

- Patients with β-thalassemia who are transfusion dependent.
- Given the seriousness of their disease, many patients affected by β-thalassemia depend on a chronic regimen of blood transfusions both for their survival and to prevent complications associated with the disease. Transfusion dependence is defined as no transfusion-free period of more than 6 weeks in the last 2 years or the transfusion of a minimum 100 mL/kg of concentrated red blood cells/year in the last 2 years[2].
- Patients with β-TDT who are followed at “Centers experienced in the treatment of beta thalassaemia” according to the EMA prescription or at "Hemoglobinopathies Reference Centers" for the countries, like Italy, where a specialized network, recognized by institutions, exists[12,13]. In consideration that not all countries have such specialist Centers we recommend that a summary of the below described information must be available.
- At Hemoglobinopathies Specialized Centers it is possible to track clinical data, particularly data related to transfusion. At these centers, the patient also has a preliminary interview during which the available therapeutic options and the proposed procedure are discussed. The transplant center will carry out the final interview and final assessment and collect the patient’s informed consent form.
- It is essential that patients are followed at a Hemoglobinopathies Specialized Center for at least 2 years and that correct registration of medical data is guaranteed, with attention to data concerning comorbidities, laboratory and instrumental test results, and blood transfusions.
- Traceability of blood transfusion data is particularly important in order to calculate annual transfusion and iron intake requirements, to register any allo-antibodies and transfusion reactions, and to evaluate the need for any further therapy after gene therapy.

Figure 1. Algorithm for the selection of transfusion-dependent β-thalassemia patients for gene therapy



PATIENT AGE

Background and discussion

It is now clear that age is a risk factor for clinical outcomes in patients with hemoglobinopathies both during conventional therapy and in a transplant setting. In the 1980s, the Pesaro Group stratified thalassemic patients aged <16 years into three different risk groups that had a significant impact on the clinical outcome after transplantation. The statistically significant factors were hepatomegaly (<2 cm versus >2 cm from the costal margin), hepatic fibrosis (absent versus present), and chelation (regular versus irregular)[4,5,14]. This last parameter was linked to the use of deferoxamine, the only chelating drug then available and importantly it reflected the concept of duration and exposure to iron overload and toxicity. However, the Pesaro Classification was not predictive of clinical outcomes in adult patients with β-TDT, probably because they had already been exposed to iron chelation for too long. The correlation between the Pesaro Classification and our current understanding of the physiopathology of iron has now been recognized and has received fresh interpretation[15,16]; in itself, it underlines the pertinence today of the observation reported almost 30 years ago. However, the limited experience of gene therapy in clinical trials to date requires the adoption of a cautious approach when using the Pesaro classification of risk for patients undergoing gene therapy until novel evidence becomes available.

Age is also considered and established by the current regulations in force. In fact, gene therapy using LentiGlobin (Blubird Bio) has received the EMA approval for patients aged >12 years with β-TDT with non-β0/β0 genotype[17].

It is important to note that the safety and efficacy of gene therapy in children aged <12 years has not been established.

Concerning children under the age of 12 they cannot be considered in this expert opinion because current EMA regulation excludes them from gene therapy because safety and efficacy of gene therapy in children aged <12 years has not been established. Children indication will require dedicated trials including a sufficient number of patients. It should be noted that the vector “GLOBE” that showed promising results in young children[18] is actually not registered by any regulatory authority worldwide.

The upper age limit for gene therapy has not been fixed as it depends on the clinical condition (comorbidities/organ damage) of each individual. It is therefore up to the physician to examine the characteristics of their patient and evaluate the clinical priorities to be addressed.

Up to now limited data are available regarding the impact of the aging process on medullary microenvironment in healthy subjects. Studies carried out in elderly subjects (aged >65 years) have shown how mobilization of CD34+ cells with granulocyte-colony stimulating factor (G-CSF) can be reduced, but always on the basis of transplant requirements19–21. In non-anemic older patients aged between 66 and 73 years, two independent studies have described a reduction in circulating CD34+ cells compared with younger subjects associated with age dependent increased serum stem cell factor, indicating an age dependent change in hematopoietic cytokine(s)22–24. Inadequate erythropoietin (EPO) production has been suggested to contribute to anemia in the elderly, but again limited and not conclusive studies are available. Furthermore, the presence of a co-morbidity such as diabetes, arterial hypertension or kidney failure might further alter the cellular response to EPO21,25. Finally, bone marrow microenvironment is dynamically changing during aging and it might represent an additional factor to be taken into consideration in identification of candidate patients for gene therapy26,27.

Recommendations

- Gene therapy products are today registered over the age of 11 years.
- Patients more than 50 years old must be considered with caution.
- We do not recommend gene therapy in patients aged over 55.

ALLOGENEIC TRANSPLANTATION

Background and discussion

The two key factors that determine eligibility for allogeneic transplantation and affect clinical outcomes after transplantation are age and the availability of an HLA-identical family donor.

Currently the European Hemoglobinopathies Registry states that allogeneic transplantation should take place in patients who are less than 14 years old. The correlation between age and clinical outcome is directly related to the iron-related organ damage that results from long-term repeated blood transfusions, despite the incredible improvements seen in treatment, iron chelation and other supportive measures.

HLA compatibility is also extremely important in the transplant setting, both because it reduces the immunological complications related to antigenic disparity that results in graft-versus-host disease (GvHD) and because it allows less ablative and immunosuppressive conditioning regimens to be used, therefore reducing the transplant procedure-related toxicity and likelihood of infection complications.

Data from large cohort studies have confirmed that an HLA-identical family donor is the donor profile that guarantees the best outcome in terms of overall survival and thalassemia-free survival. Data from the European Hemoglobinopathies Registry show that patients aged less than 14 years with an HLA-identical family donor have an overall survival rate of 91.9% and thalassemia-free survival of 86%[9,28]. Recently experiences evaluating transplantation with matched unrelated donors and haploidentical donors in patients with β-TDT have been published with relevant results[8,29,30].

Recommendations

- Patient age <14 years and HLA-identical family donor are the factors associated with the best transplant outcomes in patients with β-TDT.

GENOTYPE

A patient's β-globin genotype must be registered on presentation of a report from a National Health accredited laboratory.

The HbVar database (http://globin.cse.psu.edu) can be used to define hematologic expression (β0 o β+ forms).

At present, regulatory authorities do not request studies of alfa, delta and gamma globin genes. However, the complete molecular globin profile of a patient could be useful in assessing their expected response to therapy.

Recommendations

- Identification of a patient's complete molecular globin profile is strongly advised before starting gene therapy.

Background and discussion

The experience of the Pesaro group showed that constant monitoring of the level of iron accumulation is the determining factor for transplant outcome[10]. The Pesaro classification[14] (validated for patients <18 years only), which stratifies patients on the basis of three independent factors (hepatomegaly, hepatic fibrosis and length of exposure time to chelating therapy), remains pertinent today because it highlights the importance of monitoring iron accumulation throughout a patient's lifetime[16,31]. Moreover, an elevated LIC (as any elevated iron burden biomarker) is a sign of years of exposure to toxic forms of iron[16] and parallel chelation compliance and tissue damage.

According to the formula recently proposed by Coates[15], iron-associated tissue toxicity is due to more than a single factor: the quantity of reactive species (non-transferrin bound iron, labile plasma iron, etc.); genetic antioxidant factors (superoxide dismutase, catalase, glutathione peroxidase); environmental factors (food antioxidants, other metals such as copper, selenium); length of time exposed to the toxic effects of iron.

Recent *in vitro* studies[32,33] have shown that accumulation of iron can also influence the medullary bone marrow microenvironment and have a negative impact on both the quality and the quantity of the hematopoietic stem progenitor cells.

In relation to iron accumulation, when considering gene therapy, attention should be drawn to the paragraph in the Zynteglo® EMA product approval documentation entitled 'Risks asscіated with TDT and iron overload', section 4.4 Special warnings and precautions for use in APPENDIX I – SUMMARY OF PRODUCT CHARACTERISTICS used for gene therapy and approved by the EMA34:

EMA[34]: *'Patients with TDT experience iron overload due to chronic red blood cell (RBC) transfusions that can lead to end organ damage. HSC transplantation with myeloablative conditioning is not appropriate for patients with TDT who have evidence of severely elevated iron in the heart i.e., patients with cardiac T2* <10 ms by magnetic resonance imaging (MRI). MRI of the liver should be performed on all patients prior to myeloablative conditioning. It is recommended that patients with MRI results demonstrating liver iron concentration (LIC) ≥15 mg/g undergo liver biopsy for further evaluation. If the liver biopsy demonstrates bridging fibrosis, cirrhosis, or active hepatitis, HSC transplantation with myeloablative conditioning is not appropriate.'* Adopting a cautious approach, the SITE feels that more stringent iron accumulation criteria should be used than those indicated by the EMA (≥15 mgFe/g Liver, see above).

Recommendations

- Patients with significant iron accumulation (LIC >7 mgFe/g Liver) should have a 'suspended indication' for gene therapy until values return to acceptable limits (LIC<7 mgFe/g Liver).
- Caution should be exercised and iron levels should be strictly monitored after adequate chelation therapy has been set up.

ORGAN DAMAGE ASSESSMENT

Cardiomyopathy

Liver Disease

Endocrinopathy

Nephropathy

Thrombophilic Status

CARDIOMYOPATHY

Background and discussion

In patients with β-TDT, cardiac complications and premature death due to cardiomyopathy represent a serious problem. Iron-related cardiac damage manifests as cardiac insufficiency, arrhythmias, sudden death or progressive congestive heart failure. Overall, iron-related cardiac complications are the leading cause of death and the biggest cause of morbidity in these patients[35,36].

In patients with β-TDT, the outcome of allogeneic transplantation is directly correlated with good performance status, adequate iron chelation without iron-related organ damage, and the absence of comorbidities[37]. These same parameters also influence clinical outcomes of gene therapy in this patient population[37].

The prevalence and predictive factors for cardiac complications in patients undergoing allogeneic transplantation are not known. In clinical trials of gene therapy in patients with β-TDT, a heart T2* MRI <10 ms and clinically significant pulmonary hypertension are conditions that should exclude access to gene therapy[2]. In the light of observations on restrictive myocardiopathy in adult patients with preserved ejection fraction (EF) and without myocardial iron overload, the SITE panel consider that these factors should be included in the exclusion criteria.

Recommendations

The following heart conditions should be considered exclusion criteria:

- myocardial iron overload T2* MRI <10 ms in the previous 6 months[38,39];
- pulmonary hypertension (determined by cardiac catheterization)[40];
- serious congestive heart failure (New York Heart Association class III or above);
- significant arrhythmia requiring therapy, as defined by European Heart Rhythm Association (EHRA) guidelines[41];
- myocardial ischemia in the previous 12 months, as defined by European Society of Cardiology (ESC) guidelines[41];
- restrictive myocardiopathy, as defined by ESC guidelines[41].

Background and discussion

Follow-up data on outcomes following gene therapy are lacking. It is therefore useful to refer to clinical experience from hematopoietic stem cell transplants in this setting. In 1990, Lucarelli et al.[5] identified fibrosis and hepatomegaly as factors predictive of a negative transplant outcome and, despite the subsequent controversy concerning the definition of hepatomegaly, these original criteria are still considered valid (i.e., hepatomegaly is an expression of hepatic iron accumulation and of the duration of toxic iron exposure)[16].

Hepatic fibrosis. Hepatic fibrosis is a marker of exposure to iron and to viruses. Patients with liver disease, and particularly those with severe hepatic fibrosis or cirrhosis, are at higher risk of veno-occlusive disease (VOD) after myeloablative conditioning regimens and are also at increased risk of fatal liver failure even with reduced intensity conditioning[42]. Assessment of the level of liver fibrosis should ideally be determined for all potential gene therapy candidates before treatment is started.

Hepatitis C virus infection. Data correlating hepatitis C virus (HCV) infection with gene therapy is lacking because patients who test positive for HCV-RNA are not usually enrolled into clinical trials. It is useful, therefore, to refer to the experience from hematopoietic stem cell transplants in patients with β-TDT to guide the selection of gene therapy candidates. HCV infection (i.e., HCV-RNA positivity) appears to be clinically relevant in this setting, as hepatitis can worsen after immune reconstitution[43]. The risk of death after virus reactivation is 8% and involves the allogeneic transplant setting only. HCV-positivity is therefore generally considered to be a significant risk factor in patients with β-TDT undergoing transplantation; patients with HCV have an increased risk of death after allogeneic stem cell transplantation even if they have normal or almost normal liver function[44]. The association between HCV status and the risk of post-transplant VOD remains a subject of debate and is not supported by all experts. However, some experts consider HCV to be an independent risk factor for post-transplant VOD[45,46]. An increase in the risk for fatal VOD in HCV-positive patients who had received cyclophosphamide and >12 Gy total body irradiation has been observed, but this seems to be correlated to liver inflammation and fibrosis and to the components of the conditioning regimen rather than the HCV itself[46]. Considering the availability of effective and safe anti-viral therapies that have demonstrated excellent results also in thalassemia patients, it does not seem justified to consider HCV-RNA-positive subjects as candidates for gene therapy[47–50].

Hepatitis B virus infection. As stated previously, due to the lack of data for gene therapy in this setting, it is useful to refer to experience of hematopoietic stem cell transplant to address this issue. In patients with β-TDT who have undergone hematopoie stem cell transplant, hepatitis B virus (HBV) virus reactivation can be followed by normalization with seroconversion until HbsAg negativity is achieved in 25% of cases, even without anti-viral therapy, while fulminant hepatitis is observed in a small percentage of patients (3%). In most cases, any new acute phase of hepatitis is only light and asymptomatic with a moderate and long-lasting increase in transaminase levels[51]. The risk of reactivation of hepatitis B in HbsAg negative and anti-HBc positive subjects is around 6.5%. There are no observed differences between patients who are HBV-DNA positive and those who are HBV-DNA negative; among those with hematologic diseases, the risk of reactivation is higher in those treated with rituximab[52].

Clinical presentation of HBV reactivation ranges from asymptomatic cases to acute liver failure and death. Mortality rates are higher in those patients who do not receive anti-viral agents compared with those who do (~30% versus 12%, respectively); moreover, among those patients treated with anti-viral agents, mortality rates are lower in those treated with entecavir compared to those treated with lamivudine[53]. The use of anti-HBV prophylaxis is therefore recommended in patients who are HbsAg-negative, anti-HBc-positive with hematologic diseases, irrespective of the patient's basal anti-HBs and HBV-DNA status. In addition, treatment with 2nd-generation nucleoside analogs (e.g. entecavir/tenofovir) is strongly recommended for patients with active chronic HBV infection who are candidates for immunosuppressive therapy[54].

Recommendations

- The ideal candidates for gene therapy are patients with β-TDT who have no or only slight hepatic fibrosis, which in this context the SITE defines as level F1 based on Fibroscan® or Ishak stage 0-1-(2) based on liver biopsy.
- There are no reasons to consider anti-HCV-positive patients to be contraindicated for gene therapy if they have eliminated the virus either spontaneously or after anti-viral therapy, provided that they have no other hepatic or extra-hepatic contraindications.
- Gene therapy is contraindicated in patients with chronic HBV infection, as defined by European Association for the Study of Liver (EASL) guidelines.
- Gene therapy can be considered for patients who have occult HBV infection, as defined by EASL guidelines, provided that they accept appropriate prophylaxis and have no other contraindications to treatment.

ENDOCRINOPATHY

Background and discussion

Appropriate long-term medical treatment is the essential factor for good clinical outcomes after hematopoietic stem cell transplants[37] and this is probably also the case for patients undergoing gene therapy. Endocrinopathies (e.g. diabetes, hypogonadism, short stature, hypothyroidism) have been shown to be associated with higher ferritin levels[55], as in cases when patients started iron chelation therapy late[56], and cardiac overload[57], indicating that the presence of one or more endocrinological complications usually reflects an inadequate iron chelation therapy and therefore a high iron burden for the patient concerned[56,58].

The term 'diabetic stem cell mobilopathy' is used to indicate scarce mobilization of the bone marrow hematopoietic stem cells to the peripheral blood in patients with diabetes. This occurs because diabetes radically changes the bone marrow microenvironment resulting in a net reduction in the release of hematopoietic stem cells[59]. In clinical trials of gene therapy, the presence of an endocrinopathy has never been an exclusion criterion. However, exclusion criteria have been reported to include the statement: 'Any other evidence of severe iron overload that, in the Investigator's opinion, warrants exclusion'[2].

Endocrinological complications have not been shown to be relevant to post-transplant outcome. These should, however, always be assessed before hematopoietic stem cell transplant in order to plan adequate monitoring of these conditions post-transplant[60].

Recommendations

- A cautious approach should be adopted at this stage given the limited clinical experience with gene therapy.
- Insulin-dependent patients should not be considered high priority patients for gene therapy.

NEPHROPATHY

Background and discussion

In patients with β-TDT, kidney damage can be related either to iron overload or to chronic hypoxia caused by anemia[60]. In this setting, experience from allogeneic hematopoietic stem cell transplantation cannot be applied to the use of nephrotoxic immunosuppressors.

Different thresholds used to define chronic disease have been reported in the literature. Examples include: 'kidney disease with a calculated creatinine clearance <30% normal value'[60]; 'kidney disease with a baseline estimated glomerular filtration rate <70 mL/min/1.73 m^2 ' [2]; or 'adequate renal function as evidenced by serum creatinine < 1.5 upper limit of normal'[18].

Published clinical trials on the use of gene therapy in patients with β-TDT patients report that changes in kidney function are an exclusion criterion for clinical trial enrollment.

Recommendations

- Kidney function must be assessed before starting gene therapy.
- Patients with abnormal renal function should be considered for gene therapy with extreme caution and are not given priority at this stage.

THROMBOPHILIC STATUS

Background and discussion

Patients with β-TDT present with hypercoagulability (which is particularly evident in splenectomized patients) due to a high platelet count and peripheral erythroblastosis[60–64].

Due to peripheral stem cell mobilization and the use of G-CSF during gene therapy, patients with a history of thrombotic events must be identified and their thrombophilic status assessed before being considered eligible for gene therapy[18,65]. Clinical studies of the use of gene therapy in patients with β-TDT have confirmed that a history of thrombotic events should be an exclusion criterion for enrollment; hypersplenism should also be a contraindication as it can have a negative impact on grafting.

Assessment of/screening for pro-thrombotic status before treatment is essential in order to confirm patient suitability and to promote good outcomes from gene therapy.

Recommendations

- Patients with a 'low risk thrombophilic screen' (defined here as low levels of protein S, protein C and/or antithrombin III) are not excluded from gene therapy as these may be related to thalassemic/sickle cell liver damage[18] .
- Patients with a remote history of a thromboembolic event and no documented pulmonary hypertension can be evaluated for gene therapy after the suspension of anticoagulant therapy.
- Patients with a negative history of significant previous thrombotic events are considered eligible for enrollment[18].
- Hypersplenism is a contraindication for gene therapy[2].
- Patients with white blood cell count $<3 \times 10^9$/L, and/or platelet count $<100 \times 10^9$/L are exclusion criteria (i.e. pancytopenia post-infection)[2].
- Lupus anticoagulant (LAC) represents a contraindication to gene therapy; an uncorrected bleeding disorder (e.g. low levels of Factor VII or VIII) is also an exclusion to treatment.

SUMMARY OF RECOMMENDATIONS

Patient selection must be carried out through a consensus decision between the Center of the Network of Hemoglobinopathies and the treatment center qualified to carry out HSC transplantation.

Gene therapy should be restricted to centers experienced with myeloablative conditioning and with the treatment of transfusion-dependent β-thalassemia. Patients selected for gene therapy must fulfill the inclusion criteria established for LentiGlobin European Medicines Authority (EMA) license and be accredited by the Foundation for the Accreditation of Cellular Therapy and Joint Accreditation Committee (FACT-JACIE) for allo-transplant.

It is the opinion of the expert panel that patients with β-TDT who could represent possible candidates for gene therapy with the only product currently available that is approved by the EMA (EMA/CHMP/166977/2019) (Zynteglo®) must satisfy the criteria listed in the summary table below.

Patients must be considered unsuitable for gene therapy if they:

- meet exclusion criteria indicated by the regulatory authorities (see EMA/CHMP/166977/2019)
- have uncontrollable iron overload and/or chronic organ damage (e.g. pulmonary hypertension).

Controllable iron overload requires reassessment. Caution must be exercised when assessing patients with complications and comorbidities.

Access to gene therapy must be reassessed according to scientific and regulatory updates.

In recognition of the continuous evolution of medical scientific knowledge, of new data being presented in the literature and, in specific cases, of changes to recommendations from the regulatory authorities, the SITE and EHA expert panel will update this algorithm and the supporting notes when new evidence is published that could modify the strength of the current recommendations. As these technologies will likely improve over time, the inclusion or change in some parameter may happen; for example, more effective vectors (β^0 patients), less myeloablation or non-toxic regimens, novel medications that improve some of the conditions related to iron overload.

Should no such evidence emerge, the SITE and EHA expert panel will review and update the algorithm and supporting notes every 2 years.

High priority patients	Ineligible patients	Assessable patients undergoing ongoing changes to therapy (i.e. potential future candidates for gene therapy)
• patients followed by specialized hemoglobinopathies centers • β-TDT patients aged >12 and <55 years • patients eligible for allogeneic transplant with no HLA-identical family donor • patients with no significant iron overload • patients with no evidence of organ damage • patients who are registered in a qualified transplant center that has experience in hematopoietic stem cell transplant and is connected to a center specialized in the treatment of patients with β-TDT • good compliance with treatment	• patients not followed by specialized hemoglobinopathies centers • patients aged <12 years and >55 years • patients with uncontrolled iron overload • patients with chronic organ damage, hepatopathy, insulin-dependent diabetes, nephropathy, positive thrombophilic status	• patients followed by specialized hemoglobinopathies centers • β-TDT patients aged >12 years and <55 years • patients with a non-β0/β0 genotype • patients eligible for allogeneic transplant with no family HLA-identical donor • patients with iron overload: LIC >7mgFe/g Liver dw – cardiac MRI T2* < 10 ms in the previous 6 months • patients with non-insulin dependent diabetes • patients with slight and/or reversible cardiopathy • patients who are HCV-RNA and/or HBV-DNA positive • good compliance with treatment

β-TDT, transfusion-dependent β thalassemia; HLA, human leukocyte antigen; HCV, hepatitis C virus; LIC, liver iron concentration; MRI, magnetic resonance imaging.

Appendix (as Supplemental material): Consideration and requirements for the identification of qualified treatment centers for gene therapy

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REQUIREMENTS AND RECOMMENDATIONS FOR THE IDENTIFICATION OF QUALIFIED TREATMENT CENTRES

1. Ability to deliver gene therapy

2. Medical experience

3. Instrumentation and organizational procedures
   - 3.1 Instrumentation
   - 3.2 Organisational procedures

Appendix (as Supplemental material): Consideration and requirements for the identification of qualified treatment centers for gene therapy

In order to facilitate the identification of qualified treatment centers potentially capable of practising gene therapy for the treatment of patients with β-TDT by the competent authorities, the SITE proposes a set of requirements and recommendations for suitable treatment centers, divided into three main categories:

1. ability to deliver therapy
2. pathology experience
3. instrumentation and organizational procedures.

Overall, these requirements and recommendations will show:

- whether a center meets current standards for hematopoietic stem cell transplants
- whether the staff/manager of the center has adequate knowledge of hemoglobinopathies and the treatment of adolescents and adults with β-TDT
- the consolidated experience of the center in conducting prospective experimental and observational clinical studies
- adherence to the CD34+ cell collection and processing methods consistent with FACT-JACIE standards (7th edition)
- that the center is compliant with risk management procedures as defined by FACT-JACIE standards (7th edition).

Moreover, centers should also provide specialists to discuss advantages (lack of GVHD, potential definitive cure, gene addition versus gene editing) and potential side effects of the treatment (lack of complete curative effects, loss of fertility and potential development of MDS or oncogenesis) to the patients and family members. Fertility experts, scientists, psychologists and social workers should be available to the patients to address all the questions.

1. Ability to deliver gene therapy

- **Each center must meet the national mandatory requirements for peripheral hematopoietic cell transplantation.**
- **Each center must be accredited according to FACT-JACIE standards (7th edition).**
  - This standard of accreditation is an indicator of the ability of the treatment center to comply with operating standards in terms of skills in transplantation and apheresis (consistent with European Society for Bone and Marrow Transplantation requirements and as included in the protocols of the manufacturing companies) to ensure the safety of the patient treated with gene therapy.
- **Each center must have a healthcare team properly trained in the correct management of gene therapies, based on gene addition technology and cell therapies (accreditation for therapy with immune effector cells according to FACT-JACIE standards (7th edition).**
  - In addition, the center must have *ad hoc* scientific training available, organized by manufacturing companies, in the language of the country where the center is located.
- **The center must have staff with documented experience in the staminoapheresis protocols involved in gene therapy.**
- **The center must have the skills to implement the long-term follow-up of patients treated with gene therapy as required by the European Medicines Agency (EMA) and therefore must have a data management service.**
  - The center and the specialized center for hemoglobinopathies will have to carry out a long-term surveillance study aimed at monitoring patient safety for 15 years following the infusion of gene therapy and will have to disseminate the relevant data.
- **The center must adhere to the FACT-JACIE standards (7th edition) and the manufacturer's quality standards.**
  - The manufacturing company will support the quality managers at the center in maintaining the required accreditation and quality standards, and this will be verified by regular audits.

2. Medical experience

- **The center has close collaboration with specialized centers for hemoglobinopathies** in order to promote better patient care and potentially to contribute to the achievement of optimal results from gene therapy**.**
- **The center must have a formally approved transplant program and perform allogeneic hematopoietic stem cell transplants in malignant and non-malignant diseases consistent with JACIE-FACT standards (7th edition).**
    - This requirement is specified to guarantee the institutional recognition of the program, the possibility of extending the procedure to patients presenting the indication, and the allocation of adequate resources for the correct management of patients with β-thalassemia. The experience in allogeneic hematopoietic stem cell transplantation primarily in non-malignant pathologies, in particular β-TDT or sickle cell anemia, is not only an indicator of the capacity of the manager/staff of the center to administer gene therapy to these patients, but also of the on-site availability of the skills necessary to perform apheresis and infusion in hemoglobinopathies according to the approved protocols. The experience is documented in the curriculum of the Program Director and/or at least one of its senior collaborators. The center must ensure adequate patient follow-up on an outpatient basis, through internal resources or through a consolidated network with a specialized center for hemoglobinopathies.
- **The center must have experience in stem cell mobilization with the use of mobilizing agents, such as filgrastim and plerixafor.**
- **The center must be able to perform stem cell collection in selected patients using central venous catheterization, possibly after sedation for insertion of the catheter.**
- **The center must have experience in the infusion of cryopreserved stem cells according to the FACT-JACIE criteria (7th edition).**

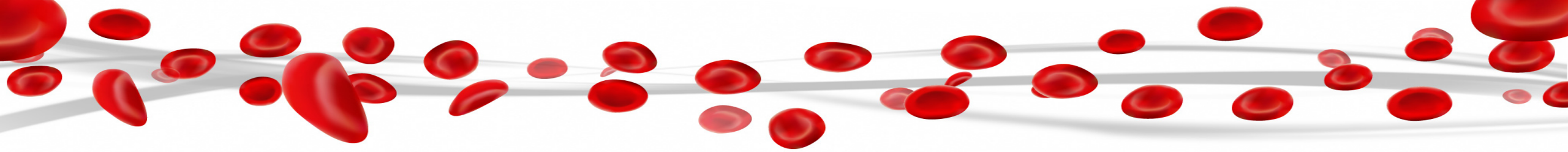
- **The center must have a procedural system which guarantees the reinfusion of the cryopreserved cells within a timeframe of <30 minutes per bag.**
- **The center has resources to make further recommendations for gene therapy.**
  - The center is aware of the possible need to extend the treatments offered in terms of dedicated space and resources for the gene therapy to ensure timely access for potentially eligible patients.
- **The center has (either internally or through agreed access) the necessary pharmacological skills to titrate treatment with myeloablative conditioning agents.**
  - Experience in the use of busulfan as a myelosuppressive agent is required.
- **The center has direct access to intensive cardiovascular care unit (CICU).**

3. Instrumentation and organizational procedures

- **3.1 Instrumentation**
- **Apheresis equipment.** The center must have one of the following specific pieces of equipment: Spectra Optia® Apheresis System, Fresenius Kabi Amicus® Separator or COBE® Spectra Apheresis System. The apheretic collection must be consistent with the requirements that guarantee the quality of the cells collected necessary to produce the gene therapy product as specified by the manufacturers**.**
- **Equipment and space for the storage of hematopoietic cells and gene therapy products.** The center must have the following:
- containers suitable for storing cells at between 1°C and 10°C, with a temperature monitoring system equipped with an audible alarm (in case cell collection by apheresis is carried out on two consecutive days).
- a device that can store the gene therapy product in liquid nitrogen in the gas phase with automatic filling with centralized temperature monitoring for the correct conservation of genetically modified organisms; furthermore, the device must be able to contain the product box used as specified by the manufacturer; sufficient space must be available to ensure adequate long-term conservation of the rescue cells and their maintenance must comply with specific procedures, so that they can be used in emergency situations.
- **Labeling and shipping.** The center must be able to create labels with the unique identification number of the donation in compliance with the FACT-JACIE standards (7th edition) and maintain operational compliance with the EU directive 2015/565 regarding the creation of the Single European Code (SEC).
- **IT systems, tools and portals for programming and traceability.** The center must have an IT system that ensures patient traceability and data collection in compliance with current regulations; the IT system must be able to integrate with the tools and portals of programming and traceability of the producing companies to guarantee the correct and safe delivery of gene therapy.

- **3.2 Organizational procedures**
- **Collection of the starting material to produce the drug.** The center must ensure that the patient's CD34+ cell collection is carried out in an apheresis center that can follow the protocols shared with the manufacturing companies, certifying their competence in performing apheretic collection and maintaining the fundamental quality standards for the collection of an adequate quantity and quality of cells to start the production of the drug.
- **Minimum experience for staminoapheresis:** it is essential that the center has performed at least 50 staminoapheresis per year in the last three consecutive years. (Note that the term ‘staminoapheresis’ refers to a patient, not the procedure.)
- The center must guarantee compliance with a schedule for the collection and shipment of hematopoietic cells functional to production within the times suggested by the manufacturer in order to ensure that the quality of the cells is preserved. In this context, it should be noted that the cells collected by apheresis have an average life of 48 hours, therefore active communication between the center and the specialized laboratory is required to ensure timely cell manipulation and production of the gene therapy product. To coordinate this, it is strongly recommended that an administrator (comparable to the CAR-T manager involved in coordinating therapy with immune effector cells) is appointed.
- The center must comply with an organizational system for the collection of the patient's hematopoietic cells, to allow a possible second apheresis on the day following the first cell collection in order to collect the minimum quantity of cells necessary for the correct execution of gene therapy.
- **Shipping of starting material for drug production.** The center must use the courier and the shipping method specified by the manufacturer for transport to and from the cell manipulation site to ensure that the quality of the cells is preserved.

- **Hospitalization.** The center must be organized in such a way as to guarantee the patient's stay after infusion of the product for 3–6 weeks in an adequate and dedicated environment to monitor correct implantation. If necessary, the center must also be able to accommodate re-admission of the patient for further follow-up procedures/tests after discharge.
- **Reporting of adverse events.** The center must be able to report any adverse events, procedural deviations, exceptions and other events relevant to the drug as per the national pharmacovigilance system and in compliance with the FACT-JACIE standards (7th edition) and those of the drug producer.
- **Welcome for patients and their families.** The center must put in place an adequate reception system for patients and their families, even if they are not of Italian nationality, using cultural mediators when necessary and providing social aid to support the patient and their family in an auxiliary infrastructure (e.g. to manage the possible housing needs of the patient’s caregivers/ family if they are of a different nationality).



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