bjh review

Modern treatment of thalassaemia intermedia

Caterina Borgna-Pignatti

Clinica Pediatrica, Università di Ferrara, Ferrara, Italy

Summary

The term thalassaemia intermedia includes a large spectrum of conditions of varying severity. Blood transfusion and chelation are necessary in some patients, especially during childhood, in order to promote growth and prevent bone deformities. Alloimunisation, however, is frequent and can be difficult to control. Splenectomy is usually needed at some time because of hypersplenism and mechanical encumbrance. Reactivation of HbF is possible only in a small proportion of patients: hydroxycarbamide (also known as hydroxyurea) appears to be the most effective drug for this purpose. Antioxidant agents, although theoretically useful, do not improve haemoglobin levels. Stem cell transplantation is an option limited to the severe forms. Gene therapy and other molecular approaches are subjects of intense study. Numerous complications, including pulmonary hypertension, thrombotic events, pseudoxanthoma elasticum and osteoporosis, have been described and all contribute to complicate the treatment of a disease that represents a significant burden for the patients and their families.

Keywords: thalassaemia intermedia; HbF reactivation; antioxidants; transfusion; hydroxycarbamide.

The term thalassaemia intermedia indicates a clinical condition of intermediate gravity between thalassaemia minor, the asymptomatic carrier, and thalassaemia major, the transfusion-dependent, severe form. The clinical picture, however can be very variable, and span from a mild anaemia with no bone abnormalities to a condition characterised by more severe anaemia (Hb in the order of 60–70 g/l), and intense erythropoietic activity. Age at presentation seems to be a good indicator of future transfusion independency (Cao, 1988). In all cases, the spleen is enlarged to a variable degree, usually from infancy or early childhood.

The genetic bases of the disease are likewise variable. The molecular pathophysiology of β-thalassaemia has recently been reviewed (Quek & Thein, 2006). In general, any imbalance between the α and β globin chains of haemoglobin can cause a thalassaemic picture. Patients affected by E/β° thalassaemia, the most common haemoglobinopathy in many Asian countries, often develop a clinical picture of intermediate gravity.

It is usually possible to make a genotype/phenotype correlation, which is of great usefulness for genetic counselling and prenatal diagnosis. However, several genetic and environmental factors can modify the clinical picture. Different genes can directly affect haemoglobin synthesis or influence the course of thalassaemia, acting on different aspects of the disease. In the first case, any factor able to reduce the degree of globin imbalance may change homozygous β-thalassaemia into a milder clinical form. On the other hand, β-thalassaemia heterozygotes can develop a more severe phenotype because of an increase in the globin chain imbalance.

Examples of external modifiers are polymorphisms that occur at loci acting outside the haemopoietic system, (on bilirubin, iron, the coagulation pathway, and, perhaps, bone metabolism) that can affect clinical expression. Among others, the inheritance of the Gilbert's syndrome [mutation of the A(TA)nTAA motif of the promoter of the bilirubin UDP-glucuronosyltransferase gene], increases the indirect bilirubin level and the risk of gallstones (Borgna-Pignatti *et al*, 2003); the inheritance of one of the haemochromatosis genes can worsen the iron overload, the co-inheritance of thrombophilic defects can increase the thromboembolic risk. The availability of good medical care is also an important factor in influencing the clinical course of the disease.

Therapeutic choices

Transfusion

The most difficult therapeutic choice that needs to be made when treating a patient with thalassaemia intermedia is whether or not to initiate a chronic transfusion program.

The decision is mainly clinical. In general, transfusion becomes necessary when the sense of well being of the patient decreases to a level inadequate to the activities of a normal life. Problems usually develop, in patients used to chronic hypoxia, for levels of Hb below 70 g/l. In addition to maintaining the

Correspondence: Prof. C. Borgna-Pignatti, Clinica Pediatrica, Dipartimento di Medicina Clinica e Sperimentale, Università di Ferrara, 44100 Ferrara, Italy. E-mail: c.borgna@unife.it

 First published online 12 June 2007
British Journal of Haematology, **138**, 291–304 doi:10.1111/j.1365-2141.2007.06654.x

haemoglobin level, the patient's general condition must be considered, particularly in children, in terms of regular growth, growth velocity, bone age, bone deformities and size of the spleen. Also, it is important to consider the consequences of withholding transfusion in terms of medullary and extramedullary hyperplasia. The mild forms of thalassaemia intermedia do not require transfusion therapy, except in special circumstances, which include infection, periods of rapid growth and pregnancy. Sometimes transfusion becomes necessary during infection-induced aplastic crises. Small epidemics due to parvovirus B19 infection have been reported (Serjeant *et al*, 1981). Heart disease is also an indication to transfusion therapy. Sometimes, a transfusion regime initiated in childhood to favour growth, can be discontinued after puberty. Conversely, some adults who have remained transfusion independent for two or more decades gradually develop severe anaemia, requiring regular blood transfusion.

When the decision to transfuse is made, the transfusion regimen should be similar to the one generally adopted for thalassaemia major. A level of pretransfusional Hb around 95–100 g/l is usually sufficient to adequately suppress the bone marrow activity, promoting better growth and decreasing iron absorption from the gut.

Several authors believe that all patients homozygous for β-thalassaemia or double heterozygous for two β-thalassaemia mutations deserve to be transfused immediately following diagnosis. The complications that accompany the more severe forms of thalassaemia intermedia, in fact, are often disabling and difficult to control, and are better prevented by regular transfusion. Another reason to start transfusion early is that, when transfusion is initiated after the first few years of life, alloimmunisation and autoimmunisation represent a significant risk. In one study, the prevalence of alloimmunisation was 21 vs. 47·5% when patients who were transfused before and after the age of 3 years were compared (Spanos *et al*, 1990). Asian patients seem to be more prone to this complication, as a consequence of donor-recipient red cell antigen mismatch and, possibly, other immunological factors. In this setting, anaemia can become extremely severe, despite the use of high dose immunosuppressive therapy. Transfusion of phenotypically matched blood for the Rh and Kell systems can be effective in preventing alloimmunisation (Singer *et al*, 2000).

Splenectomy

In untransfused or rarely transfused patients, the size of the spleen inevitably increases with time, with consequent worsening of the anaemia (which may require red blood cell transfusion), and, sometimes, neutropenia and thrombocytopenia. Splenectomy usually reverses the process, allowing discontinuation of transfusion in the majority of the patients. However, it does not usually modify the high output state and the increased pulmonary artery pressure that often characterises thalassaemia intermedia (Aessopos *et al*, 2005). The surgical approach is usually laparotomic, because, at the time of splenectomy, the size of the spleen is rarely small enough for a laparoscopic approach. Also, laparoscopic splenectomy seems to be complicated by portal thrombosis more often than laparotomic splenectomy (Ikeda *et al*, 2005).

If gallstones are present, cholecystectomy should also be performed at the time of splenectomy. Prophylactic cholecystectomy, in the absence of gallstones, could be performed at the same time, in order to avoid two surgical interventions. Alternatively, it could be performed only in patients with persistently elevated indirect bilirubin, especially if the homozygous Gilbert mutation is present.

Alternatives to traditional, surgical splenectomy have been proposed. Partial splenectomy and partial dearterialisation of the spleen have an immediate beneficial effect, but are not long-lasting (de Montalembert *et al*, 1990). Partial embolization of the spleen has been successfully performed with long-lasting results, but has sometimes led to complications and it is not widely used (Politis *et al*, 1987). These surgical approaches can be used in selected patients, when conventional splenectomy is contraindicated.

Complications following splenectomy include an increased susceptibility to infections, and thrombophilia. The theory that a residual portion of spleen protects the patient from infections and thrombotic events is tempting but still unproven.

Infections. Overwhelming postsplenectomy sepsis (OPSI) is an abrupt event that can be rapidly fatal. Thalassaemia patients are exposed to a higher infectious risk than other non-immunodeficient patients splenectomized for different causes (Hansen & Singer, 2001). The most frequently responsible bacteria are *Streptococcus pneumoniae*, *Hemophilus influenzae*, *Neisseria meningitidis*, *Klebsiella*, *Escherichia coli* and *Staphylococcus aureus*.

The pathogenicity of these bacteria is further increased by the immune dysfunction induced by iron overload. Several mechanisms have been proposed, the most important of which seems to be the inhibitory effect of iron on the activity of interferon-γ. As a consequence, iron-loaded macrophages lose the ability to kill intracellular pathogens via the interferon-γ-mediated pathways. Some of this loss of ability is related to the reduced formation of nitric oxide in the presence of iron (Vento *et al*, 2006).

Recommendations by the British Committee of Standard in Haematology for the prevention of postsplenectomy infections were updated in 2002 (Davies *et al*, 2002). They include antibiotic prophylaxis with penicillin, amoxicillin or erythromycin for the first 2 years after surgery and for children up to the age of 16 years, as well as early antibiotic treatment for fever and malaise. Compliance with antibiotic prophylaxis can be a problem and requires continuous reinforcement (Borgna-Pignatti *et al*, 1984). Polysaccharidic antipneumococcal immunisation should be given 2 weeks before the procedure, or whenever possible after it. Re-immunisation is recommended every 5 years.

Immunisation against *Hemophilus influenzae* and the conjugated vaccine against serogroup *C. meningococci* are also recommended. Asplenic patients should be offered annual influenza vaccination.

Thromboembolic complications. Thromboembolic complications are frequent in thalassaemia, particularly in the intermediate form. In an Italian multicentre study, 4% of the patients with thalassaemia major, but 10% of those with thalassaemia intermedia experienced a thromboembolic event (Borgna-Pignatti *et al*, 1998).

In a report on 8860 thalassaemia patients from the Mediterranean countries and Iran, thromboembolism occurred four times more frequently in thalassaemia intermedia than in thalassaemia major, with more venous events occurring in thalassaemia intermedia and more arterial events occurring in thalassaemia major (Taher *et al*, 2006). Cappellini *et al* (2000a) reported a high prevalence of venous thrombotic events (29%) in splenectomized patients with thalassaemia intermedia. These authors and others (Eldor & Rachmilewitz, 2002) have suggested that the presence of a chronic hypercoagulable state could be due to the procoagulant effect of the anionic phospholipids exposed on the surface of the damaged circulating red blood cells.

No therapy has been demonstrated to completely prevent these thrombotic complications. However, platelet anti-aggregation agents in patients with thrombocytosis and low molecular weight heparin followed by long-term oral anticoagulants in patients with a history of thrombosis seem reasonable choices. Anti-coagulant prophylaxis appears to be warranted also before surgery and during pregnancy. Blood transfusion might reduce the thrombotic risk by diluting the procoagulant thalassaemic red cells.

Chelation

Iron overload, a major problem in thalassaemia major, is usually less severe a concern in thalassaemia intermedia, unless the patient is regularly transfused.

Iron introduced by transfusion accumulates and damages cells and tissues. Although the exact mechanism of tissue damage remains unclear, iron-induced peroxidative injury to the phospholipids of lysosomes and mitochondria, produced by free hydroxyl radicals, is believed to be the most important pathogenetic factor.

In contrast, when the patient is not, or only rarely, transfused, the iron accumulation is mainly derived from haemolysis and from intestinal absorption of the metal. It has been demonstrated that the hepatic peptide hepcidin, normally regulated by marrow activity and by iron load, is disproportionately low in individuals with thalassaemia, allowing iron to be absorbed from the gut even in the presence of severe overload (Kattamis *et al*, 2006). In thalassaemic mice the iron transporter ferroportin has been found to have a fundamental role in sustaining iron accumulation (Gardenghi *et al*, 2007). Studies performed by magnetic resonance have shown that, in thalassaemia intermedia, the iron tends to accumulate in the liver, while the heart is usually spared. Iron chelating agents are necessary to prevent or decrease the consequences of haemosiderosis. Three chelating drugs are currently available: deferoxamine, administered subcutaneously at a median dose of 40 mg/kg over 8–12 h; Deferiprone an oral product administered orally at the dose of 75–100 mg/kg/d in three subdoses given 1 h before meals; Deferasirox, also an oral formulation, has recently been approved for use by the Food and Drug Administration and by the European Agency for the Evaluation of Medicinal Products (EMEA). The recommended dosage is 10-30 mg/kg in one morning dose. All three drugs are quite effective and the choice for each patient must be made on the basis of the severity of iron overload, major site of iron deposition (if the information is available), convenience of administration, side effects and cost. The orally active chelators seem to be more efficient in gaining access to the chelatable iron pools of cardiomyocytes, binding labile iron, and attenuating reactive oxygen species formation (Glickstein *et al*, 2006). An extensive review of iron chelators has been recently published (Hershko, 2006). In the rare patients with sufficiently high haemoglobin levels, phlebotomy could be used to decrease the iron overload accumulated with previous transfusions or through the gastrointestinal tract.

Haemoglobin F reactivation

The clinical picture of thalassaemia intermedia could be greatly improved by an even partial decrease of the non-α/α globin chain imbalance. Several drugs have been tried in an attempt to reactivate γ-chain synthesis.

5-azacytidine. More than 25 years ago, studies performed in anaemic baboons demonstrated that 5-azacytidine, a cytidine analogue, was capable of increasing haemoglobin F (HbF) to levels 6 to 30 times higher than those produced by bleeding alone (DeSimone *et al*, 1982). These results suggested that the drug could have therapeutic implications in the haemoglobinopathies and opened the way to a completely new therapeutic strategy.

As a consequence, 5-azacytidine was investigated as an HbF inducer in a thalassaemia patient. This drug, when incorporated into newly synthesised DNA, leads to extensive demethylation and to reactivation of γ-globin synthesis (Ley *et al*, 1982). Small clinical trials have since been conducted but the concern about potential toxicity of this drug prevented its further use.

Hydroxycarbamide. Hydroxycarbamide, also known as hydroxyurea, an S-phase-specific and non-DNA-hypomethylating chemotherapeutic agent is capable of inducing HbF synthesis. The effect of hydroxycarbamide and other antimetabolites on HbF synthesis is mainly mediated by their cytotoxic properties. In fact, they preferentially kill

dividing cells, permitting the emergence of primitive erythroid progenitors more highly committed to HbF synthesis. Hydroxycarbamide may also have a more general role in increasing globin synthesis (Atweh & Loukopoulos, 2001).

Hydroxycarbamide has been approved by the Food and Drug Administration for use in sickle cell disease, but not in thalassaemia. However, around the world, the drug has been administered to thalassaemia patients according to many different regimens, alone or in combination with other drugs. Large series of thalassaemia intermedia patients have been recently reported from Iran (Karimi *et al*, 2005) and India (Dixit *et al*, 2005; Panigrahi *et al*, 2005) (Table I). The results were impressive, especially in the first study, where most patients were reported to have become transfusion independent. In patients who were not transfused, the haemoglobin concentration increased. Previous studies from Europe had documented a constant increase of the erythrocyte volume and in HbF, but only a modest effect on total haemoglobin concentration. α-deletions, the XmnI polymorphism (Panigrahi *et al*, 2005) and Hb E/β-thalassaemia (Singer *et al*, 2005) may be predictive of a good response to hydroxycarbamide. The improved sense of well-being, almost universally reported, may reflect the significant decrease of ineffective erythropoiesis (Loukopoulos *et al*, 1998) It has been suggested that the efficacy of treatment could decrease over time (Mancuso *et al*, 2006).

The safety of long-term therapy with hydroxycarbamide is still debated. A recent review examining the risk of leukaemic transformation in essential thrombocythaemia found a low frequency and a long latency in patients treated with hydroxycarbamide only (Chim *et al*, 2005). However, a few reports of acute myeloid leukaemia in sickle cell anaemia treated with hydroxycarbamide have been published (Wilson, 2000).

Erythropoietin. Trials of recombinant human erythropoietin (rHuEPO) for the treatment of thalassaemia were performed after studies in baboons, thalassaemic mice and in erythroid cultures demonstrated an increase in γ-globin chain synthesis and an improvement in erythropoietic parameters (Al-Khatti *et al*, 1987). In β-thalassaemic mice with sustained muscle secretion of erythropoietin obtained by gene transfer, a stable correction of anaemia was maintained for at least a year. In humans, a significant, dose-dependent increase in thalassaemic erythropoiesis, without an increase in HbF, mean corpuscular volume and mean haemoglobin content, and without a change in the α/non-α ratio has been observed, mainly in splenectomized patients with thalassaemia intermedia (Bohl *et al*, 2000). The fear that rHuEPO could further expand the erythroid mass was not substantiated. Some of the trials published on the use of rHuEPO are summarised in Table II.

The most commonly used dose of rHuEPO (500 U/kg × 3/week) is 5–10 times higher than the dose used for the anaemia of chronic renal failure. It is not clear if the simultaneous administration of iron, essential in patients with renal failure, is necessary. Often thalassaemia patients have significant iron stores, but iron could be unavailable for haematopoiesis. Long-

Table I. Hydroxycarbamide results in thalassemia intermedia.

Reference	No. of patients	HU dose	Length of therapy	Results
Mancuso *et al* (2006)	18 splenectomised untransfused	Median dosage 14·6 mg/kg; range 5–30 mg/kg	1 year	Average ↑ Hb 15 g/l; 11/18 ↑ Hb > 10 g/l
Karimi *et al* (2005)	163 I group (receving regular blood transfusion) II group (no transfusion or long-interval transfusion)	8–12 mg/kg/d	6 years	149 responded I group: 83/106 became transfusion-free; 23 had 1–2 transfusions II group: 16/43 became transfusion-free: 27 developed acceptable Hb levels
Dixit *et al* (2005)	37	10–20 mg/kg	Range: 4–36 months	46% became transfusion-free and Hb ↑ >20 g/l in 24% Hb ↑10–20 g/l and ↓ transfusion need by 50%
Bradai *et al* (2003)	7 (2 with TI)	Range 15–20 mg/kg/d	Range 13–21 months	All patients ↑ total Hb in the first month of treatment. In TI patients Hb level rose from 65 to 105 g/l.
Gamberini *et al* (2004)	6	1 g/d	90 d	↓ the size of extramedullary erythropoietic masses and cured leg ulcers
De Paula *et al* (2003)	7	10–20 mg/kg/d	6 months	3/7 ↑ Hb level of 13, 19 and 20 g/l
Cianciulli *et al* (2000)	1	20 mg/kg/d	6 months	Hb ↑ from 71 to 103 g/l
Hoppe *et al* (1999)	5	3–10 mg/kg/d	Length depends on toxicity effects of HC	Hb ↑ 30 g/l in 2; ↑ 10–20 g/l in 2; no response in 1

Hb, haemoglobin; HC, hydrocarbamide.

Table II. rHuEPO results in thalassemia intermedia.

Reference	No. of patients	Dose	Duration	Results
Chaidos *et al* (2004)	10 patients (5 with TM and 5 with TI)	150 IU/kg s.c. 3/week	At least 12 weeks	In 5 patients ↓ transfusion requirement; in 3 untransfused patients ↑ Hb; slight ↑ of HbF; ↑ sTfR
Olivieri *et al* (1992)	3 patients with TI	300–1000 U/kg s.c. 3/week	Mean period of 18 weeks	In 2 patients ↑ Hb
Bourantas *et al* (1997)	4 patients with tranfusion-dependent TI	500 U/kg s.c. 3/week		In 4 patients ↑ Hb (25 g/l); in 1 patient ↑ HbF; 3 patients transfusion-independent; in 1 patient ↓ transfusion requirement
Nisli *et al* (1996)	10 patients with TI	500–1000 U/kg s.c. 3/week	3 months	In 8 patients ↑ Hb (20 g/l); ↑ Hct; ↑ reticulocytes
Dore *et al* (1996)	8 patients with TI (6 untransfused and 2 transfused)	50 U/kg s.c. 3/week	3 months in untransfused; 6 months in transfused	In 5 untransfused patients ↑ sTfR; in 2 transfused patients ↑ sTfR, ↑ reticulocytes, ↓ transfusion requirement

TM, thalassaemia major; TI, thalassaemia intermedia; sTfR, soluble transferrin factor receptor; Hb, haemoglobin; Hct, haematocrit.

acting darbepoetin alpha, an erythropoiesis-stimulating protein with a longer serum half-life than recombinant human erythropoietin, was found to be effective in increasing the Hb levels in HbE/β-thalassaemia disease (Singer *et al*, 2003).

In conclusion, rHuEPO can increase the Hb level in a subset of patients with thalassaemia intermedia, but the effect is transient, the drug is expensive and the subcutaneous administration is inconvenient. The indication for its use is therefore very limited. The efficacy of darbepoetin-α, allowing less-frequent administration, could be helpful in some patients with HbE/β-thalassaemia disease.

Butyrate derivatives. The observation that the γ- to β-globin gene (*HBG1/2* to *HBB*) switch was delayed in infants of diabetic mothers prompted a series of experiments that demonstrated that butyric acid, which is increased in such infants, delayed or prevented globin gene switch in cultured cord blood erythroid progenitors (Perrine *et al*, 1987) and in fetal lambs (Perrine *et al*, 1988). Butyrate and butyrate derivatives are short chain fatty acids that inhibit the histone deacetylases and are believed to increase *HBG1/2* expression by increasing histone acetylation at the promoter level or by increasing the efficiency of translation of *HBG1/2* mRNA (Weinberg *et al*, 2005). Butyrate derivatives, such as arginine butyrate, sodium isobutyramide and sodium phenylbutyrate, have been tried in patients with thalassaemia intermedia. The first compound to enter a clinical trial, arginine butyrate, was reported to be effective in some patients when administered intravenously. Unfortunately, the majority of treated patients continued to suffer from anaemia. It was not possible to predict which patients would respond to therapy, on the basis of baseline HbF, type of mutation or other parameters. The oral derivatives, sodium phenylbutyrate and sodium isobutyramide, are difficult to administer because of the large number of pills that need to be given and the poor taste of the compound. Some studies reported an increase of ≥10 g/l Hb in half of the patients (Dover, 1998). In another study (Cappellini *et al*, 2000b), sodium isobutyramide was given to 12 patients with thalassaemia intermedia for 28 d. Little or no increase in the non-α/α ratio and in the percentage of HbF was observed.

Haemin. The ferric chloride salt of haem, haemin, when combined with erythropoietin, preferentially increased the production of HbF in human erythroid cells. The mechanism of action may include effects on globin gene transcription and post-translational events. Unfortunately, a pilot study performed on a small number of patients did not give satisfactory clinical results (Fibach *et al*, 1995).

Combination therapy. The idea of combining different HbF stimulating agents is very attractive and it is rationally based on the different mechanisms of action of the therapeutic agents investigated to date. Combination therapy was shown to be synergistic in different animal models (Pace *et al*, 1994). Unfortunately, the few clinical trials reported have not been completely successful. The combination of hydroxycarbamide with rHuEPO was effective in some patients, while the addition of sodium phenylbutyrate had no effect (Hoppe *et al*, 1999). In general, better responders were splenectomized, had a higher baseline HbF level and higher soluble transferrin factor receptor and erythropoietin levels. In one study (Loukopoulos *et al*, 1998), the combination of a very high dose of rHuEPO (50 000 IU three times a week) with standard dose hydroxycarbamide for 12 weeks produced an increase in HbF and total Hb levels, but these results were not maintained when the dose of rHuEPO was reduced.

Antioxidants

Oxidative damage is believed to be one of the main contributors to cell injury and tissue damage in thalassaemia. It is, at least in part, generated by the presence on the cells of free globin chains and labile plasma iron (LPI), a redox-active and chelatable component of non transferrin-bound iron. LPI can be measured using fluorescent probes (Esposito *et al*, 2003) and can be reduced by treatment with iron chelating agents.

It has been demonstrated that, in thalassaemia patients, reactive oxygen species play an important role also in the oxidative state of platelets, leading to platelet activation and potentially favouring thromboembolism (Amer & Fibach, 2004). Similarly, the chronic stress on neutrophils can reduce their antibacterial capacity and their respiratory burst response (Amer & Fibach, 2005). Treatment with antioxidants, alone or in combination, is thought to neutralise the deleterious effects of the reactive oxygen species (Rund & Rachmilewitz, 2000).

Vitamin E appears to play a prominent role in the resistance of low-density lipoproteins (LDL) to oxidation. In a study of 30 patients with thalassaemia intermedia the plasma and LDL content of α-tocopherol were significantly lower than in controls and LDL susceptibility to oxidation was enhanced. In addition, the resistance of LDL from thalassaemia patients to oxidation was strongly correlated with Vitamin E content (Tesoriere *et al*, 1998). Supplementation with Vitamin E is therefore often suggested. Data demonstrating its efficacy in improving the antioxidant/oxidant balance in plasma, LDL, and red blood cells are limited to a series of 15 patients with thalassaemia intermedia treated with Vitamin E at the dose of 600 mg/d for 9 months (Tesoriere *et al*, 2001).

N-acetylcysteine is a protein antioxidant and a radical scavenger that improves glutathione levels in oxidised erythrocytes (Pace *et al*, 2003). It is able to inhibit inducible nitric oxide synthesis, suppress cytokine expression/release and inhibit the expression of adhesion molecule and nuclear factor-κB (Caglikulekci *et al*, 2006). Its use in thalassaemia intermedia patients has been shown to ameliorate the platelets' oxidative stress and to reduce the hypercoagulability state (Amer & Fibach, 2004).

Several substances of plant origin, including rutin (vitamin P) and flavonoids, have therapeutic potential in thalassaemia. Signs of reduced oxidative damage were observed *in vivo* on the erythrocytes of a mouse model of β-thalassaemia intermedia treated with the semi-synthetic flavonoid 7- monohydroxyethylrutoside (de Franceschi *et al*, 2004). Tea polyphenols, major components of both green and black tea bind to ferric iron and could protect erythrocytes from oxidation (Rund & Rachmilewitz, 2000).

The dietary supplement *indicaxanthin* has been found to enhance, in a dose-dependent fashion, the resistance to haemolysis of human β-thalassaemic erythrocytes exposed *in vitro* to oxidative haemolysis by cumene hydroperoxide. In addition, it prevented lipid and haemoglobin oxidation, and retarded vitamin E and glutathione depletion (Tesoriere *et al*, 2006). It has been reported that the combined use of a lipid antioxidant (Vitamin E) with a protein antioxidant (*N*-acetyl cysteine) and an iron chelator (deferiprone) could be more effective than the administration of a single antioxidant (Pace *et al*, 2003).

Despite all their *in vitro* effects on erythrocytes, however, these antioxidants have not yet been shown to improve the anaemia of thalassaemia patients.

Other supplements

Vitamin C. Haemosiderotic patients are often found to be ascorbate deficient. However, ascorbate, a natural reducing agent, accelerates iron-induced lipid peroxidation in biological systems at low concentrations and it has been shown to alter the function of rat myocardial cells in culture. Supplementation with Vitamin C, therefore, is not recommended except in transfusion-dependent patients with demonstrated deficiency, on treatment with deferoxamine. In fact, deferoxamine-induced iron excretion is enhanced by ascorbic acid, which expands the chelatable pool (Nienhuis, 1981).

Folic acid. Folic acid is a co-enzyme for many important biochemical reactions including synthesis of purines, pyrimidines and nucleoproteins. The recommended daily allowance of folic acid is 65–200 μg/d for infants and children. Folic acid deficiency has been reported in both thalassaemia major and minor (Danel *et al*, 1983; Ortuno *et al*, 1990), as a consequence of increased folate utilisation caused by the increased erythropoiesis. Daily supplementation with 1 mg of folic acid is advised for patients with thalassaemia intermedia (Mojtahedzadeh *et al*, 2006).

Zinc. Serum zinc levels, but not zinc binding capacity, have been found to be low in patients with thalassaemia intermedia (Arcasoy *et al*, 2001). Zinc supplementation may become necessary during intense chelation.

Stem cell transplantation

Stem cell transplantation is an accepted treatment modality for thalassaemia major, but it has rarely been used in patients with thalassaemia intermedia. The transplant-related mortality, even in the best of conditions, averages 5%, a risk that it is worth running probably only in severe transfusion-dependent disease. An attractive alternative could be to perform transplants after reduced-intensity conditioning. Attempts have been performed to obtain a persistent stable mixed chimaerism that might be sufficient to correct an anaemia of intermediate gravity. However, the results have been inconsistent to date (reviewed in Locatelli, 2006). At least two patients with thalassaemia intermedia who had developed red blood cell auto/alloimmunisation were successfully transplanted, one

from an human leucocyte antigen-identical sibling and one from an unrelated donor with complete correction of both the congenital and the acquired defect. (De Stefano *et al*, 1999 and P. De Stefano, personal communication)

Molecular therapies and future perspectives

Several innovative therapeutic approaches to thalassaemia have been proposed, which still need to be tried clinically. They were recently reviewed in detail (Quek & Thein, 2006).

Gene therapy. The transfer of a globin gene in autologous haematopoietic stem cells is an attractive treatment possibility. It poses challenges in terms of controlling transgene expression, which ideally should be erythroid-specific, differentiation- and stage-restricted, elevated, position independent and sustained over time (Sadelain, 2006). Retroviral vectors carrying the human β-globin cassette were notoriously unstable and expressed poorly.

Lentiviral vectors and a phase I/II clinical trial. Considerable progress has now been made using lentiviral vectors (LVs), which stably transmit the β-globin expression cassette, offering new prospects for the manipulation of haemopoietic stem cells (Chang & Sadelain, 2007). Mouse studies have shown correction of the β-thalassaemia-intermedia phenotype (Malik *et al*, 2005). Recent success in the long-term correction of mouse models in human β-thalassaemia and sickle cell anaemia by lentiviral vectors, and evidence of high gene transfer and expression in transduced human haematopoietic cells, have led to a first clinical trial of gene therapy for β-thalassaemia. The LentiGlobin vector is self-inactivating and contains large elements of the β-globin locus control region as well as chromatin insulators and other features that should prevent untoward events (Bank *et al*, 2005).

Co-regulation of globin transgene expression and RNA interference. This is a combination of two methods that modulate gene function, which may be used to induce the expression of a normal haemoglobin gene while inhibiting the expression of a mutant gene. It has been used to treat sickle cell anaemia by co-regulation of globin transgene expression and RNA interference (RNAi), providing proof of principle for therapeutic strategies that require synergistic gene addition and gene silencing in stem cell progeny (Samakoglu *et al*, 2006). The technique may possibly be used in thalassaemia patients to express haemoglobin-transduced genes and silence mutant genes.

Globin gene transfer by homologous recombination. Homologous DNA recombination offers the possibility to directly correct the mutation in the patient's gene after transfer of a normal gene, avoiding the potential problem of insertional mutagenesis from a viral vector. The feasibility of the approach has been recently demonstrated by correcting haematopoietic stem cells *ex vivo* in sickle cell anaemia mice (Chang *et al*, 2006), injection into mice blastocysts and expression of the corrected gene in adult mice (Wu *et al*, 2006). These experiments offer proof of principle that gene therapy by homologous recombination in *ex vivo* embryonic stem cells is a possibility for the haemoglobinopathies (Quek & Thein, 2006). The current restrictions on human embryonic stem cell research, however, hamper investigation of this strategy (Sadelain, 2006).

Gene transfer of the hepcidin gene (HAMP). Recent studies have evaluated a gene transfer system to test whether constitutive expression of *HAMP* in thalassaemic animals might prevent increased iron absorption (Breda *et al*, 2005). Either gene transfer or pharmacological administration of hepcidin could be a useful adjunct to the control of haemosiderosis.

Therapeutic antisense mRNA. Antisense oligonucleotides targeted at aberrant splice sites can restore correct splicing in erythroleukaemic cell lines. The use of so-called morpholino oligonucleotides has enabled correction of transcribed mutant β-globin RNA. These studies have implications for the treatment of HbE/β-thalassaemia (Suwanmanee *et al*, 2002).

α-haemoglobin stabilising protein. The α-haemoglobin stabilising protein (AHSP) binds free α-globin chains, limiting the oxidative effects of α-Hb and prevents its precipitation. In humans, it is directly regulated by GATA-1. mRNA of the gene encoding AHSP (*ERAF*) has been found to be low in some patients with severe β-thalassaemia (Galanello *et al*, 2003). Upregulation of AHSP protein or the synthesis of an AHSP mimic to chaperone the free redundant α-globin in β-thalassaemia represent potential molecular therapies.

In the future, pharmacogenomics, and the so-called proteomic and metabolomic studies will also be of help in improving treatment and in characterising β-thalassaemia. In fact, we still do not know, among other things, why some patients but not others respond to HbF-inducing drugs, why some develop severe side effects from chelating agents, or why some patients who are genotypically heterozygous or homozygous behave, phenotypically, like thalassaemia intermediates.

Treatment of complications

Heart. Heart complications are the first cause of death in thalassaemia major and are the consequence of iron overload. Patients with thalassaemia intermedia who do not usually have severe haemosiderosis are less prone to cardiac problems. Nevertheless, a large multicentre study of 110 patients with a mean age of 32·5 ± 11·4 years found that 5·4% had congestive heart failure, 34% had chronic pericardial changes, and more than half had some kind of valvular problem (Aessopos *et al*, 2001).

In addition, 59% of the patients had signs of pulmonary hypertension, a cause of secondary right heart failure (Aessopos *et al*, 2001).

An Italian study comparing patients with thalassaemia intermedia and thalassaemia major found a more pronounced left ventricular remodelling in thalassaemia intermedia and emphasised the primary ethiopathogenetic role of chronically high cardiac output (Bosi *et al*, 2003). The authors suggested early transfusion and chelation in order to prevent these complications. The beneficial effects of the angiotensin-converting enzyme inhibitor enalapril in improving systolic and diastolic function have been demonstrated by echocardiography in asymptomatic or minimally symptomatic patients with β-thalassaemia major (Karvounis *et al*, 2001). No similar data have been reported for patients with thalassaemia intermedia.

Pulmonary hypertension. Pulmonary hypertension is defined as systolic pulmonary artery pressure >25 mmHg at rest and >30 mmHg during exercise with a normal pulmonary artery wedge pressure <15 mmHg and an increased pulmonary vascular resistance to greater than three Wood units. It appears that pulmonary hypertension can be associated with all kinds of chronic haemolytic anaemias (Vichinsky, 2004). The pathogenesis of the complication seems to include vasoconstriction, vessel wall hypertrophy, hypercoagulability, local thrombosis, increased thromboxane and endothelin, and decreased prostacycline and nitric oxide (Reviewed in Aessopos & Farmakis, 2005). Platelets may have an important role, as pro-coagulant, by increasing platelet release of serotonin (contributing to smooth muscle vasoconstriction and proliferation), vascular endothelial growth factor and platelet-derived growth factor (Gabbay *et al*, 2007).

Therapeutic strategies of pulmonary hypertension are still controversial. Some authors suggested that the development of pulmonary hypertension could be prevented, by starting transfusion and chelation therapy early in life for patients with thalassaemia intermedia (Aessopos *et al*, 2001). Correction of hypercoagulability and amelioration of pulmonary arterial hypertension by chronic blood transfusion was seen in an asplenic haemoglobin E/β-thalassaemia patient (Atichartakarn *et al*, 2004).

Hydroxycarbamide may significantly reduce post-splenectomy thrombocytosis and erythroblastosis, important determinants in the development of pulmonary hypertension. The use of antiplatelet agents has also been suggested. Several therapies have long been available for the treatment of primary and secondary pulmonary hypertension and are still of use (diuretics, oxygen therapy, digoxin for arrhythmias or ventricular dilatation, anticoagulants). Prior to the advent of new specific strategies, calcium channel blockers were considered the most effective therapies for pulmonary hypertension.

New and more effective pharmacotherapies have been introduced, beginning with prostacyclin analogs (epoprostenol, iloprost, oral beraprost) and, more recently, endothelin receptor antagonists (bosentan, sitaxsentan) and phosphodiesterase 5 inhibitors (sildenafil). Bosentan is the first and only approved endothelin receptor antagonist for the treatment of pulmonary hypertension.

In a recent case report, a patient with β-thalassaemia intermedia complicated by chronic pulmonary thromboembolism and liver iron overload was treated with bosentan, which improved his respiratory status without worsening his hepatopathy (Pierre *et al*, 2006). However, some patients discontinue bosentan because of hepatotoxicity or inadequate efficacy.

Sildenafil citrate is a potent inhibitor of cyclic guanosine monophosphate-specific phosphodiesterase-5, which leads to selective smooth muscle relaxation.

In thalassaemia intermedia, the drug was first successfully used in a 34-year-old transfusion-dependent man (Littera *et al*, 2002) and, more recently, in four patients who experienced reduction of pulmonary pressure, improvement of cardiovascular function and a better exercise tolerance (Derchi *et al*, 2005).

Further studies including larger groups of patients are necessary to establish the long-term safety and efficacy of these new treatments in patients with β-thalassaemia intermedia.

Erythropoietic masses. The chronic anaemia of thalassaemia intermedia, with the associated hypoxia and defective haemoglobin unloading from fetal haemoglobin, increases the bone marrow haemopoietic activity, frequently leading to extramedullary erythropoiesis. Masses, more commonly intrathoracic and sometime accompanied by haemotorax, were found in 65% of adult patients subjected to computed tomography scan (Dore *et al*, 1992).

Spinal cord compression causing paraparesis and cauda equina syndrome are emergency situations that require rapid intervention to prevent irreversible neurological damage. Hypertransfusion is effective, but regression of the erythropoietic masses can be slow (Chehal *et al*, 2003). A more rapid response can be obtained with the use of radiotherapy or with the administration of hydroxycarbamide (Cianciulli *et al*, 2000; Gamberini *et al*, 2004). Surgery has also been used in selected cases (Pornsuriyasak *et al*, 2006).

Bone abnormalities and osteoporosis. The bone abnormalities that are typical of thalassaemia major are often present in thalassaemia intermedia and can be even more marked, as a consequence of the enhanced medullary haematopoiesis. Transfusion, if started before the abnormalities have developed, prevents them. In young children, adequate transfusion at Hb levels high enough to suppress the bone marrow activity, can induce regression of already established deformities. A child with thalassaemia intermedia who underwent bone marrow transplantation for severe autoimmune anaemia gradually experienced reshaping of the facial bones previously deformed by the intense haematopoiesis (De Stefano *et al*, 1999). Plastic and dental

surgery can be an option in severe deformities that affect the quality of life of the patient (Jurkiewicz *et al*, 1967; Gotte *et al*, 2001).

Bone mineral density is almost uniformly reduced. Fractures due to minor traumas may also signal medullary overgrowth and, when frequent, suggest the need for transfusion. In a study from North America, the prevalence of fractures in thalassaemia intermedia patients was 12%. It increased with age and with the use of sex hormone replacement (Vogiatzi *et al*, 2005). Bone and joint pains are a frequent complaint. 25-OH vitamin D deficiency has been reported (Napoli *et al*, 2006).

Vitamin D and calcium are given to patients with thalassaemia intermedia according to different regimens, in the hope of improving mineral density. However, recent results from the Women's Health Initiative study failed to demonstrate a reduction in hip fracture during daily supplementation with 1000 mg of elemental calcium as calcium carbonate and 400 IU of vitamin D3. On the other hand, the risk of kidney stones increased by 17%. If these results were extrapolated to thalassaemia patients, one should conclude that the prescribed dose of vitamin D should be higher, in the order of 700–800 IU (Deane *et al*, 2007) and calcium should be given at 1200–1500 mg/d, as part of the diet or as supplements (Rosen, 2005). Additional therapy is probably necessary in patients at risk of fractures.

Also, particular attention should be made in monitoring urinary calcium excretion in thalassaemia patients who already have an increased risk of kidney stones.

Additional therapy should include biphosphonates, potent inhibitors of osteoclast activation. Alendronate, pamidronate and zoledronic acid have all been shown to be effective in improving bone mineral density, modifying the biochemical markers of bone formation and resorption, and reducing pain (Voskaridou *et al*, 2006). Dental surveillance is necessary during treatment with biphosphonates, because several cases of jaw necrosis have been described (Migliorati *et al*, 2006). Other treatments have reduced the risk of fracture, including raloxifene, teriparatide (Hodsman *et al*, 2006), or strontium ranelate, (Meunier, 2001), however only a few have been tried in thalassaemia.

Leg ulcers. Leg ulcers over the medial malleolus are a common and distressing problem in thalassaemia intermedia. Their pathogenesis has been related to: hypoxia caused by chronic anaemia, abnormal rheology of the thalassaemic red cells, venous stasis and high HbF concentration.

Treatment is often unsatisfactory, as leg ulcers are difficult to heal and frequently recur. It includes pressure dressing, skin grafting, blood transfusion, hydroxycarbamide, arginine butyrate, granulocyte colony-stimulating factor, rHuEPO, platelet growth factor and local hyperbaric oxygen chamber (reviewed in Aessopos *et al*, 2006).

In one report, a transfusion-independent patient suffering from persistent leg ulcerations responded to 1-year therapy with exchange transfusions, which reduced the percentage of HbF from 70 to 35%. (Aessopos *et al*, 2006)

Endocrine glands and pregnancy. Endocrine problems are less common in thalassaemia intermedia than in thalassaemia major, but the frequency reported is highly variable, according to the severity of the anaemia and the iron overload. Hypogonadism is the most frequent endocrine complication, affecting female more than male patients (Papadimas *et al*, 2002), followed by diabetes and hypothyroidism, reported in 24% and 5·7% of thalassaemia intermedia patients respectively. Delayed puberty is not unusual, as are irregular menses.

The early recognition and treatment of endocrinopathies is very important to prevent late complications and increase the chances of successful reproduction. Fertility is usually normal. In a large series of patients with thalassaemia intermedia and normal menstrual cycles, most pregnancies were achieved spontaneously, while a few followed induction either by insemination or *in vitro* fertilisation (Skordis *et al*, 1998). Pregnancy, however, is often burdened with complications. Intrauterine growth restriction is observed in more than half of cases (Nassar *et al*, 2006). Anaemia becomes more severe during pregnancy, especially during the first and second trimester and, in order to prevent fetal growth restriction due to hypoxia, blood transfusion is often required, with the consequent risk of antibody formation in previously untransfused women.

Successful correction of the anaemia was obtained in two patients with the administration of erythropoietin (Lialios *et al*, 2000; Bennett *et al*, 2005). On the contrary, the development of anomalies in animal models treated with hydroxycarbamide limit its use in pregnancy (Woo *et al*, 2004).

Splenomegaly can interfere with the enlargement of the uterus and can be complicated by hypersplenism. Splenectomy can therefore become necessary during gestation or after delivery. Antithrombotic therapy is suggested before delivery because of the hypercoagulable state (Cappellini *et al*, 2000a). Caesarean section is often chosen as the modality of delivery.

The physiological haemodynamic changes and the increased cardiac output require constant monitoring of the heart function. Only careful medical care during this time of delicate equilibriums can ensure a pregnancy outcome with minimal adverse effects. Hypogonadotropic hypogonadism can be treated by the administration of conjugated oestrogens and progesterone to females and testosterone enanthate to males.

Pseudoxanthoma elasticum. A syndrome characterised by typical skin lesions, angioid streaks in the retina, calcified arterial walls, aortic valve disease was described for the first time in patients with thalassaemia in 1992 (Aessopos *et al*, 1992). It is due to a diffuse elastic tissue defect and it is undistinguishable from inherited pseudoxanthoma elasticum. The genetic defect of the inherited form, however, was not found in thalassaemia patients with the disorder (Hamlin *et al*,

2003). It is age-dependent and it is more common in thalassaemia intermedia than in thalassaemia major. The pathophysiology is unclear and it has been attributed to iron-induced oxidative damage. Its progression is complicated by thrombotic and haemorragic events similar to those of the inherited form (Cianciulli *et al*, 2002).

No effective therapy is available. The results of a recent trial of aluminium hydroxide, given with the rational of pharmacologically limiting the intestinal absorption of phosphate, demonstrated the histopathologic regression of skin calcification in some patients. If this effect could be demonstrated also for eye and vascular lesions, aluminium hydroxide could represent the first real treatment option for pseudoxanthoma elasticum (Sherer *et al*, 2005).

Platelet antiaggregants could be helpful for thrombosis prevention, but the haemorragic risk, mainly at the gastrointestinal or intracranial level, precludes their use.

Liver. Gallstones are more common in thalassaemia intermedia than in thalassaemia major because of greater ineffective erythropoiesis and peripheral haemolysis.

Hepatocellular carcinoma represents a frequent complication in patients with liver cirrhosis either secondary to genetic haemochromatosis or to chronic viral hepatitis. As a consequence of the numerous risk factors present in thalassaemia patients, the development of the tumour is to be expected. In fact, a survey has identified several such cases, more frequently in patients with thalassaemia intermedia than major, at a median age of 45 ± 11 years (Borgna-Pignatti *et al*, 2004). Prevention requires scrupulous treatment of liver iron overload, and antiviral therapy of chronic hepatitis C virus (HCV) and hepatitis B virus (HBV) infection. Periodical abdominal ultrasonography and α-fetoprotein monitoring are advised, especially in patients with one or more risk factors.

Secondary gout. High serum levels of uric acid are not unusual in thalassaemia patients, especially when massive extramedullary haematopoiesis is present. Gouty arthritis, however, has been so rarely reported (Paik *et al*, 1970; Kumar & Gruber, 2003) that the use of allopurinol does not seem necessary.

Conclusions

Given the high variability of the clinical picture of thalassaemia intermedia, no standardised treatment can be proposed. Therapeutic measures must be tailored for each patient according to the severity of the disease and the spectrum of complications.

Transfusion should be considered early for patients whose genotype suggests a severe clinical course, provided that the blood supply is safe and chelation is available. Splenectomy is usually necessary at some point, but great attention should be taken in prevention and early diagnosis of infections and thromboembolic events. Twenty years after their introduction, HbF-stimulating agents remain an unmet promise, except for a small subset of patients. Hydroxycarbamide is the more effective of these agents and it should be tried in selected conditions. Unfortunately, no clear indications exist to predict which patients will respond. Likewise, the theoretical benefits of antioxidants, often demonstrated *in vitro*, have not yet been confirmed by clinical trials. Stem cell transplantation is limited to severe cases and gene therapy, although actively pursued, is still in a preclinical phase. Treatment of the many complications of thalassaemia intermedia is improving with increased understanding of their pathophysiology, but longer survival is accompanied by the appearance of new complications that continue to challenge our ability to treat this protean disease.

Future perspectives include gene therapy research with lentivirus vectors, co-regulation of globin transgene expression and silencing the mutant genes, and homologous DNA recombination techniques. Proteomic studies will extend the level of analysis of nucleic acids to the final gene expression products and their functional networks. New personalised therapies will hopefully emerge from the pharmacogenomic analysis of individual biologic variation in drug metabolism and efficacy.

References

Aessopos, A. & Farmakis, D. (2005) Pulmonary hypertension in beta-thalassemia. *Annals NewYork Academy of Sciences*, **1054,** 342–349.

Aessopos, A., Savvides, P., Stamatelos, G., Rombos, J., Tassiopoulos, T., Karagiorga, M., Kaklamanis, P. & Fessas, P. (1992) Pseudoxanthoma elasticum-like skin lesion and angiod lesions and angiod streaks in beta-thalassemia. *American Journal of Hematology*, **41,** 159–164.

Aessopos, A., Farmakis, D., Karagiorga, M., Voskaridou, E., Loutradi, A., Hatziliami, A., Joussef, J., Rombos, J. & Loukopoulos, D. (2001) Cardiac involvement in thalassemia intermedia: a multicenter study. *Blood*, **97,** 3411–3416.

Aessopos, A., Farmakis, D., Deftereos, S., Tsironi, M., Polonofi, A., Moyssakis, I., Diamanti-Koudaraki, E. & Papalambros, E. (2005) Cardiovascular effects of splenomegaly and splenectomy in beta-thalassemia. *Annals of Hematology*, **84,** 353–357.

Aessopos, A., Kati, M., Tsironi, M., Polonifi, E. & Farmakis, D. (2006) Exchange blood transfusions for the treatment of leg ulcerations in thalassemia intermedia. *Haematologica*, **91**(5 Suppl.), ECR11.

Al-Khatti, A., Veith, R.W., Papayannopoulou, T., Fritsch, E.F., Goldwasser, E. & Stamatoyannopoulos, G. (1987) Stimulation of fetal hemoglobin synthesis by erythropoietin in baboons. *New England Journal of Medicine*, **317,** 415–420.

Amer, J. & Fibach, E. (2004) Oxidative status of platelets in normal and thalassemic blood. *Thrombosis and Haemostasis*, **92,** 1052–1059.

Amer, J. & Fibach, E. (2005) Chronic oxidative stress reduces the respiratory burst response of neutrophils from beta-thalassemia patients. *British Journal of Haematology*, **129,** 435–441.

Arcasoy, A., Canata, D., Sinav, B., Kutlay, L., Oguz, N. & Sen, M. (2001) Serum zinc levels and zinc binding capacity in thalassemia. *Journal of Trace Elements in Medicine and Biology*, **15,** 85–87.

Atichartakarn, V., Chuncharunee, S., Chandanamattha, P., Likittanasombat, K. & Aryurachai, K. (2004) Correction of hypercoagulability

and amelioration of pulmonary arterial hypertension by chronic blood transfusion in an asplenic hemoglobin E/beta-thalassemia patient. *Blood*, **103,** 2844–2846.

Atweh, G.F. & Loukopoulos, D. (2001) Pharmacological induction of fetal hemoglobin in sickle cell disease and beta- thalassemia. *Seminars in Hematology*, **38,** 367–373.

Bank, A., Dorazio, R. & Leboulch, P. (2005) A phase I/II clinical trial of the beta-globin gene therapy for beta-thalassemia. *Annals of the New York Academy of Sciences*, **1054,** 308–316.

Bennett, M., Macri, C.J. & Bathgate, S.L. (2005) Erythropoietin use in a Jehovah's witness with anemia and beta-thalassemia: a case report. *The Journal of Reproductive Medicine*, **50,** 135–137.

Bohl, D., Bosch, A., Cardona, A., Solvetti, A. & Heard, J.M. (2000) Improvement of erythropoiesis in beta-thalassemic mice by continuous erythropoietin delivery from muscle. *Blood*, **95,** 2793–2798.

Borgna-Pignatti, C., De Stefano, P., Barone, F. & Concia, E. (1984) Penicillin compliance in splenectomized thalassemics. *European Journal of Pediatrics*, **142,** 83–85.

Borgna-Pignatti, C., Carnelli, V., Caruso, V., Dore, F., De Mattia, D., Di Palma, A., Di Gregorio, F., Romeo, M.A., Longhi, R., Mangiagli, A., Melevendi, C., Pizzarelli, G. & Musumeci, S. (1998) Thromboembolic events in beta thalassemia major, an Italian multicenter study. *Acta Haematologica*, **99,** 76–79.

Borgna-Pignatti, C., Rigon, F., Merlo, L., Chakrok, R., Micciolo, R., Perseu, L. & Galanello, R. (2003) Thalassemia minor, the Gilbert mutation, and the risk of gallstones. *Haematologica*, **88,** 1106–1109.

Borgna-Pignatti, C., Vergine, G., Lombardo, T., Cappellini, M.D., Cianciulli, P., Maggio, A., Renda, D., Lai, M.E., Mandas, A., Forni, G., Piga, A. & Bisconte, M.G. (2004) Hepatocellular carcinoma in the thalassaemia syndromes. *British Journal of Haematology*, **124,** 114–117.

Bosi, G., Crepaz, R., Gamberini, M.R., Fortini, M., Scarcia, S., Bonsante, E., Pitscheider, W. & Vaccari, M. (2003) Left ventricular remodelling, and systolic and diastolic function in young adults with beta thalassaemia major: a Doppler echocardiographic assessment and correlation with haematological data. *Heart*, **89,** 762–766.

Bourantas, K., Economou, G. & Georgiou, J. (1997) Administration of high doses of recombinant human erythropoietin to patients with beta-thalassemia intermedia: a preliminary trial. *European Journal of Haematology*, **58,** 22–25.

Bradai, M., Abad, M.T., Pissard, S., Lamraoui, F., Skopinski, L. & de Montalembert, M. (2003) Hydroxyurea can eliminate transfusion requirements in children with severe beta-thalassemia. *Blood*, **102,** 1529–1530.

Breda, L., Gardenghi, S., Guy, E., Rachmilewitz, E.A., Weizer-Stern, O., Adamsky, K., Amariglio, N., Rechavi, G., Giardina, P.J., Grady, R.W. & Rivella, S. (2005) Exploring the role of hepcidin, an antimicrobial and iron regulatory peptide, in increased iron absorption in beta-thalassemia. *Annals of the New York Academy of Sciences*, **1054,** 417–422.

Caglikulekci, M., Dirlik, M., Pata, C., Plasse, M., Tamer, L., Ogetman, Z. & Ercan, B. (2006) Effect of N-acetylcysteine on blood and tissue lipid peroxidation in lipopolysaccharide-induced obstructive jaundice. *Journal of Investigative Surgery*, **19,** 175–184.

Cao, A. (1988) Diagnosis of beta-thalassemia intermedia at presentation. *Birth Defects Original Article Series*, **23,** 219–226.

Cappellini, M.D., Graziadei, G., Ciceri, L., Comino, A., Bianchi, P., Porcella, A. & Fiorelli, G. (2000a) Oral isobutyramide therapy in patients with thalassemia intermedia: results of a phase II open study. *Blood Cells Molecules & Disease*, **26,** 105–111.

Cappellini, M.D., Robbiolo, L., Bottasso, B.M., Coppola, R., Fiorelli, G. & Mannucci, A.P. (2000b) Venous thromboembolism and hypercoagulability in splenectomized patients with thalassaemia intermedia. *British Journal of Haematology*, **111,** 467–473.

Chaidos, A., Makis, A., Hatzimichael, E., Tsiara, S., Gouva, M., Tzouvara, E. & Bourantas, K.S. (2004) Treatment of beta-thalassemia patients with recombinant human erythropoietin: effect on transfusion requirements and soluble adhesion molecules. *Acta Haematologica*, **111,** 189–195.

Chang, A.H. & Sadelain, M. (2007) The genetic engineering of hematopoietic stem cells: the rise of lentiviral vectors, the conundrum of the LTR, and the promise of lineage-restricted vectors. *Molecular Therapy*, **15,** 445–456.

Chang, J.C., Ye, L. & Kan, Y.W. (2006) Correction of the sickle cell mutation in embryonic stem cells. *Proceedings of the National Academy of Sciences of the United States of America*, **103,** 1036–1040.

Chehal, A., Aoun, E., Koussa, S., Skoury, H., Koussa, S. & Taher, A. (2003) Hypertransfusion: a successful method of treatment in thalassemia intermedia patients with spinal cord compression secondary to extramedullary hematopoiesis. *Spine*, **28,** E245.

Chim, C.S., Kwong, Y.L., Lie, A.K., Ma, S.K., Chan, C.C., Wong, L.G., Kho, B.C., Lee, H.K., Sim, J.P., Chan, C.H., Chan, J.C., Yeung, Y.M., Law, M. & Liang, R. (2005) Long-term outcome of 231 patients with essential thrombocythemia: prognostic factors for thrombosis, bleeding, myelofibrosis, and leukemia. *Archives of Internal Medicine*, **165,** 2651–2658.

Cianciulli, P., di Toritto, T.C., Sorrentino, F., Sergiacomi, L., Massa, A. & Amadori, S. (2000) Hydroxyurea therapy in paraparesis and cauda equina syndrome due to extramedullary haematopoiesis in thalassaemia: improvement of clinical and haematological parameters. *European Journal of Haematology*, **64,** 426–429.

Cianciulli, P., Sorrentino, F., Maffei, L., Amadori, S., Cappabianca, M.P., Foglietta, E., Carnevali, E. & Pasquali-Ronchetti, I. (2002) Cardiovascular involvement in thalassemic patients with Pseudoxanthoma elasticum-like skin lesions. A long term follow up study. *European Journal of Clinical Investigation In Press*, **32,** 700–706.

Danel, P., Girot, R. & Tchernia, G. (1983) Thalassemia major manifested by megaloblastic anemia caused by folate deficiency. *Archives Francaises de Pediatrie*, **40,** 799–801.

Davies, J.M., Barnes, R. & Milligan, D. (2002) Update of guidelines for the prevention and treatment of infection in patients with an absent or dysfunctional spleen. *Clinical Medicine*, **2,** 440–443.

De Paula, E.W., Lima, C.S., Arruda, W.R., Alberto, F.L., Saad, S.T. & Costa, F.F. (2003) Long-term hydroxyurea in beta-thalassemia patients. *European Journal of Haematology*, **70,** 151–155.

De Stefano, P., Zecca, M., Giorgiani, G., Perotti, C., Giraldi, E. & Locatelli, F. (1999) Resolution of immune haemolytic anaemia with allogeneic bone marrow transplantation after an unsuccessful autograft. *British Journal of Haematology*, **106,** 1063–1064.

Deane, A., Constancio, L., Fogelman, I. & Hampson, G. (2007) The impact of vitamin D status on changes in bone mineral density during treatment with bisphosphonates and after discontinuation following long-term use in post-menopausal osteoporosis. *BMC Musculoskeletal Disorders*, **8,** 3. doi:10.1186/1471-2474-8-3.

Derchi, G., Forni, G.L., Formisano, F., Cappellini, M.D., Galanello, R., D'Ascola, G., Bina, P., Magnano, C. & Lamagna, M. (2005) Efficacy and safety of sildenafil in the treatment of severe pulmonary

hypertension in patients with hemoglobinopathies. *Haematologica*, **90,** 452–458.

DeSimone, J., Heller, P., Hall, L. & Zwiers, D. (1982) 5-Azacytidine stimulates fetal hemoglobin synthesis in anemic baboons. *Proceedings of the National Academy of Sciences of the United States of America*, **79,** 4428–4431.

Dixit, A., Chatterjee, T.C., Mishra, P., Choudhrey, D.R., Mahapatra, M., Tyagi, S., Kabra, M., Saxsena, R. & Choudrey, W.P. (2005) Hydroxyurea in thalassemia intermedia-a promising therapy. *Annals of Hematology*, **84,** 441–446.

Dore, F., Cianciulli, P., Rovasio, S., Oggiano, L., Bonfigli, S., Murineddu, M., Pardini, S., Simonetti, G., Gualdi, G., Papa, G. & Longinotti, M. (1992) Incidence and clinical study of ectopic erythropoiesis in adult patients with thalassemia intermedia. *Annali Italiani di Medicina Interna*, **7,** 137–140.

Dore, F., Bonfigli, S., Gaviano, E., Pardini, S. & Longinotti, M. (1996) Serum tranferrin receptor levels in patients with thalassemia intermedia during rHuEPO administration. *Haematologica*, **81,** 37–39.

Dover, G.J. (1998) Hemoglobin switching protocols in thalassemia: experience with sodium phenylbutyrate and hydroxyurea. *Annals of the New York Academy of Science*, **850,** 80–86.

Eldor, A. & Rachmilewitz, E.A. (2002) The hypercoagulable state in thalassemia. *Blood*, **99,** 36–43.

Esposito, B.P., Breuer, W., Sirankapracha, P., Pootrakul, P., Hershko, C. & Cabantchik, Z.I. (2003) Labile plasma iron in iron overload: redox activity and susceptibility to chelation. *Blood*, **102,** 2670–2677.

Fibach, E., Kollia, P., Schechter, A.N., Noguchi, C.T. & Rodgers, G.P. (1995) Hemin-induced acceleration of hemoglobin production in immature cultured erythroid cells: preferential enhancement of fetal hemoglobin. *Blood*, **85,** 2967–2974.

de Franceschi, L., Turrini, F., Honczarenko, M., Ayi, K., Rivera, A., Fleming, M.D., Law, T., Mannu, F., Kuypers, F.A., Bast, A., van der Vijgh, W.J. & Brugnara, C. (2004) In vivo reduction of erythrocyte oxidant stress in a murine model of beta-thalassemia. *Haematologica*, **89,** 1287–1298.

Gabbay, E., Reed, A. & Williams, T.J. (2007) Assessment and treatment of pulmonary arterial hypertension: an Australian perspective in 2006. *Internal Medicine Journal*, **37,** 38–48.

Galanello, R., Perseu, L., Giagu, N., Sole, G. & Perra, C. (2003) AHSP expression in beta thalassemia carriers with thalassemia intermedia phenotype. *Blood*, **102,** 1881 (abstract).

Gamberini, M.R., Fortini, M. & De Sanctis, V. (2004) Healing of leg ulcers with hydroxyurea in thalassaemia intermedia patients with associated endocrine complications. *Pediatric Endocrinology Reviews*, **2**(Suppl. 2)**,** 319–322.

Gardenghi, S., Marongiu, M.F., Ramos, P., Guy, E., Breda, L., Chadburn, A., Liu, Y., Amariglio, N., Rechavi, G., Rachmilewitz, E.A., Breuer, W., Cabantchik, Z.I., Wrighting, D.M., Andrews, N.C., de Sousa, M., Giardina, P.J., Grady, R.W. & Rivella, S. (2007) Ineffective erythropoiesis in beta-thalassemia is characterized by increased iron absorption mediated by down-regulation of hepcidin and up-regulation of ferroportin. *Blood*, [Epub ahead of print] doi:10·1182/blood-2006-09-048868.

Glickstein, H., El, R.B., Link, G., Breuer, W., Konijn, A.M., Hershko, C., Nick, H. & Cabantchik, Z.I. (2006) Action of chelators in iron-loaded cardiac cells: accessibility to intracellular labile iron and functional consequences. *Blood*, **108,** 3195–3203.

Gotte, P., Consolo, U., Faccioni, F. & Bertoldi, C. (2001) Associated orthodontic, surgical and hematological management of Cooley s anemia. Report of a case. *Minerva Stomatologica*, **50,** 47–54.

Hamlin, N., Beck, K., Baccelli, B., Cianciulli, P., Pasquali-Ronchetti, I. & Le Saux, O. (2003) Acquired pseudoxanthoma elasticum-like syndrome in beta-thalassemia patients. *British Journal of Haematology*, **122,** 852–854.

Hansen, K. & Singer, D.B. (2001) Asplenic-hyposplenic overwhelming sepsis, postsplenectomy sepsis revisited. *Pediatric and Developmental Pathology*, **4,** 105–121.

Hershko, C. (2006) Oral iron chelators: new opportunities and new dilemmas. *Haematologica*, **91,** 1307–1312.

Hodsman, A., Papaioannou, A. & Cranney, A. (2006) Clinical practice guidelines for the use of parathyroid hormone in the treatment of osteoporosis. *CMAJ*, **175,** 48.

Hoppe, C., Vichinsky, E., Lewis, B., Foote, D. & Styles, L. (1999) Hydroxyurea and sodium phenylbutyrate therapy in thalassemia intermedia. *American Journal of Hematology*, **62,** 221–227.

Ikeda, M., Sekimoto, M., Takiguchi, S., Kubota, M., Ikenaga, M., Yamamoto, H., Fujiwara, Y., Ohue, M., Yasuda, T., Imamura, H., Tatsuta, M., Yano, M., Furukawa, H. & Monden, M. (2005) High incidence of thrombosis of the portal venous system after laparoscopic splenectomy: a prospective study with contrast-enhanced CT scan. *Annals of Surgery*, **241,** 208–216.

Jurkiewicz, M.J., Pearson, H.A. & Furlow, L.T. (1967) Surgical reconstruction of the maxilla in severe thalassemia. *Plastic and Reconstructive Surgery*, **39,** 459–464.

Karimi, M., Darzi, H. & Yavarian, M. (2005) Hematologic and clinical responses of thalassemia intermedia patients to hydroxyurea during 6 years of therapy in Iran. *Journal of Pediatric Hematology/Oncology*, **27,** 380–385.

Karvounis, H.I., Zaglavara, T.A., Parharidis, G.E., Nouskas, I.G., Hassapopoulou, E.P., Gemitzis, K.D. & Louridas, G.E. (2001) An angiotensin-converting enzyme inhibitor improves left ventricular systolic and diastolic function in transfusion-dependent patients with beta-thalassemia major. *American Heart Journal*, **141,** 281.

Kattamis, A., Papassotiriou, I., Palaiologou, D., Apostolakou, F., Galani, A., Ladis, V., Sakellaropoulos, N. & Papanikolaou, G. (2006) The effects of erythropoietic activity and iron burden on hepcidin expression in patients with thalassemia major. *Haematologica*, **91,** 809–812.

Kumar, V. & Gruber, B. (2003) Tophaceous gout in a patient with thalassemia. *Journal of Clinical Rheumatology*, **9,** 280–284.

Ley, T.J., DeSimone, J., Anagnou, N.P., Keller, G.H., Humphries, R.K., Turner, T.H., Young, N.S., Keller, P. & Nienhuis, A.W. (1982) 5-Azacytidine selectively increases gamma-globin synthesis in a patients with beta+-thalassemia. *New England Journal of Medicine*, **307,** 1469–1475.

Lialios, G., Makrydimas, G., Tsanadis, G., Lolis, D. & Bourantas, K. (2000) Effective treatment of beta-thalassemia intermedia during pregnancy with rHuEpo. A case report. *Minerva Ginecologica*, **52,** 29–31.

Littera, R., La Nasa, G., Derchi, G., Cappellini, M.D., Chang, C.Y. & Contu, L. (2002) Long-term treatment with sildenafil in a thalassemic patient with pulmonary hypertension. *Blood*, **100,** 1516–1517.

Locatelli, F. (2006) Reduced-intensity regimens in allogeneic hematopoietic stem cell transplantation for hemoglobinopathies. *Hematology/American Society of Hematology Education Program*, 398–401.

Loukopoulos, D., Voskaridou, E., Stamoulakatou, A., Papassotiriou, Y., Kalotychou, V., Loutradi, A., Cozma, G., Tsiarta, H. & Pavlides, N. (1998) Hydroxyurea therapy in thalassemia. *Annals of the New York Academy of Sciences*, **850,** 120–128.

Malik, P., Arumgam, P.I., Yee, J.K. & Puthenveetil, G. (2005) Successful correction of the human Cooley's anemia beta-thalassemia major phenotype using a lentiviral vector flanked by the chicken hypersensitive site 4 chromatin insulator. *Annals of the New York Academy of Sciences*, **1054,** 238–249.

Mancuso, A., Maggio, A., Renda, D., Di Marzo, R. & Rigano, P. (2006) Treatment with hydroxycarbamide for intermedia thalassaemia: decrease of efficacy in some patients during long-term follow up. *British Journal of Haematology*, **133,** 105–106.

Meunier, P.J. (2001) Anabolic agents for treating postmenopausal osteoporosis. *Joint Bone Spine*, **68,** 576–581.

Migliorati, C.A., Siegel, M.A. & Elting, L.S. (2006) Bisphosphonate-associated osteonecrosis: a long-term complication of bisphosphonate treatment. *Lancet Oncology*, **7,** 508–514.

Mojtahedzadeh, F., Kosaryan, M., Mahdavi, M.R. & Akbari, J. (2006) The effect of folic acid supplementation in beta-thalassemia major: a randomized placebo-controlled clinical trial. *Archives of Iranian Medicine*, **9,** 266–268.

de Montalembert, M., Girot, R., Revillon, Y., Jan, D., Adjrad, L., Ardjoun, F.Z., Belhani, M. & Najean, Y. (1990) Partial splenectomy in homozygous beta thalassaemia. *Archives of Disease in Childhood*, **65,** 304–307.

Napoli, N., Carmina, E., Bucchieri, S., Sferrazza, C., Rini, G.B. & Di Fede, G. (2006) Low serum levels of 25-hydroxy vitamin D in adults affected by thalassemia major or intermedia. *Bone*, **38,** 888–892.

Nassar, A.H., Usta, I.M., Rechdan, J.B., Koussa, S., Inati, A. & Taher, A.T. (2006) Pregnancy in patients with beta-thalassemia intermedia: outcome of mothers and newborns. *American Journal of Hematology*, **81,** 499–502.

Nienhuis, A.W. (1981) Vitamin C and iron. *New England Journal of Medicine*, **304,** 170–171.

Nisli, G., Kavakli, K., Vergin, C., Oztop, S. & Cetingul, N. (1996) Recombinant human EPO trial in T.I. *Journal of Tropical Pediatrics*, **42,** 330–334.

Olivieri, N.F., Freedman, M.H., Perrine, S.P., Dover, G.J., Sheridan, B., Essentine, D.L. & Nagel, R.L. (1992) Trial of recombinant human erythropoietin: three patients with thalassemia intermedia. *Blood*, **80,** 3258–3260.

Ortuno, F., Remacha, A., Martin, S., Soler, J. & Gimferrer, E. (1990) Prevalence of folate deficiency in beta and delta-beta heterozygous thalassemia. *Haematologica*, **75,** 585.

Pace, B., Li, Q., Peterson, K. & Stamatoyannopoulos, G. (1994) Alpha-amino butyric acid cannot reactivate the silenced gamma gene of the beta locus YAC transgenic mouse. *Blood*, **84,** 4344–4353.

Pace, B.S., Shartava, A., Pack-Mabien, A., Mulekar, M., Ardia, A. & Goodman, S.R. (2003) Effects of N-acetylcysteine on dense cell formation in sickle cell disease. *American Journal of Haematology*, **73,** 26–32.

Paik, C.H., Alavi, I., Dunea, G. & Weiner, L. (1970) Thalassemia and gouty arthritis. *The Journal of the American Medical Association*, **213,** 296–297.

Panigrahi, I., Dixit, A., Arora, S., Kabra, M., Mahapatra, M., Choudhry, V.P. & Saxena, R. (2005) Do alpha deletions influence hydroxyurea response in thalassemia intermedia? *Hematology*, **10,** 61–63.

Papadimas, J., Goulis, D.G., Mandala, E., Georgiadis, G., Zournatzi, V., Tarlatzis, B.C. & Bontis, J.N. (2002) Beta-thalassemia and gonadal axis: a cross-sectional, clinical study in a Greek population. *Hormones (Athens)*, **1,** 179–187.

Perrine, S.P., Miller, B.A., Greene, M.F., Cohen, R.A., Cook, N., Shackleton, C. & Faller, D.V. (1987) Butyric acid analogues augment gamma globin gene expression in neonatal erythroid progenitors. *Biochemical and Biophysical Research Communications*, **148,** 694–700.

Perrine, S.P., Rudolph, A., Faller, D.V., Roman, C., Cohen, R.A., Chen, S.J. & Kan, Y.W. (1988) Butyrate infusions in the ovine fetus delay the biologic clock for globin gene switching. *Proceedings of the National Academy of Sciences of the United States of America*, **85,** 8540–8542.

Pierre, B., Maillot, F., Casset-Sedon, D., Regina, S., Sitbon, O. & Cosnay, P. (2006) Chronic pulmonary thromboembolism revealing beta-thalassemia intermedia after splenectomy. *La Revue de Medecine Interne*, **27,** 950–953.

Politis, C., Spigos, D.G., Georgiopoulou, P., Vrettou, H., Economidou, I., Germenis, A.E., Richardson, C. & Papaevangelou, G. (1987) Partial splenic embolization for hypersplenism of thalassaemia major, five-year follow up. *British Medical Journal*, **294,** 665–667.

Pornsuriyasak, P., Suwatanapongched, T., Wangsuppasawad, N., Ngodngamthaweesuk, M. & Angchaisuksiri, P. (2006) Massive hemothorax in a beta-thalassemic patient due to spontaneous rupture of extramedullary hematopoietic masses: diagnosis and successful treatment. *Respiratory Care*, **51,** 272–276.

Quek, L. & Thein, S.L. (2006) Molecular therapies in β-thalassaemia. *British Journal of Haematology*, **136,** 353–365.

Rosen, C.J. (2005) Clinical practice. Postmenopausal osteoporosis. *New England Journal of Medicine*, **353,** 595–603.

Rund, D. & Rachmilewitz, E. (2000) New trends in the treatment of beta-thalassemia. *Critical Reviews in Oncology/Hematology*, **33,** 105–118.

Sadelain, M. (2006) Recent advances in globin gene transfer for the treatment of beta-thalassemia and sickle cells anemia. *Current Opinion in Hematology*, **13,** 142–148.

Samakoglu, S., Lisowski, L., Budak-Alpdogan, T., Usachenko, Y., Acuto, S., Di Marzo, R., Maggio, A., Zhu, P., Tisdale, J.F., Riviere, I. & Sadelain, M. (2006) A genetic strategy to treat sickle cell anemia by coregulating globin transgene expression and RNA interference. *Nature Biotechnology*, **24,** 89–94.

Serjeant, G.R., Topley, J.M., Mason, K., Serjeant, B.E., Pattison, J.R., Jones, S.E. & Mohamed, R. (1981) Outbreak of aplastic crises in sickle cell anaemia associated with parvovirus-like agent. *Lancet*, **2,** 595.

Sherer, D.W., Singer, G., Uribarri, J., Phelps, R.G., Sapadin, A.N., Freund, K.B., Yanuzzi, L., Fuchs, W. & Lebwohl, M. (2005) Oral phosphate binders in the treatment of pseudoxanthoma elasticum. *Journal of the American Academy of Dermatology*, **53,** 610–615.

Singer, S.T., Wu, V., Mignacca, R., Kuypers, F.A., Morel, P. & Vichinsky, E.P. (2000) Alloimmunization and erythrocyte autoimmunization in transfusion-dependent thalassemia patients of predominantly Asian descent. *Blood*, **96,** 3369–3373.

Singer, S.T., Sweeters, N., Vichinsky, E.P., Wagner, A.J. & Rachmilewitz, E.A. (2003) A dose-finding and safety study of darbopoietin alfa (erythropoiesis stimulating protein) for the treatment of anemia in patients with thalassemia intermedia. *Blood*, **102,** 268a.

Singer, S.T., Kuypers, F.A., Olivieri, N.F., Weatherall, D.J., Mignacca, R., Coates, T.D., Davies, S., Sweeters, N. & Vichinsky, E.P. E/beta Thalassaemia Study Group (2005) Fetal haemoglobin augmentation in E/beta(0) thalassaemia: clinical and haematological outcome. *British Journal of Haematology*, **131,** 378–388.

Skordis, N., Christou, S., Koliou, M., Pavlides, N. & Angastiniotis, M. (1998) Fertility in female patients with thalassemia. *Journal of Pediatric endocrinology & metabolism*, **11,** 935–943.

Spanos, T., Karageorga, M., Ladis, V., Peristeri, J., Hatziliami, A. & Kattamis, C. (1990) Red cell alloantibodies in patients with thalassemia. *Vox Sanguinis*, **58,** 50–55.

Suwanmanee, T., Sierakowska, H., Fucharoen, S. & Kole, R. (2002) Repair of a splicing defect in erythroid cells from patients with beta-thalassemia/HbE disorder. *Molecular Therapy*, **6,** 718–726.

Taher, A., Isma'eel, H., Mehio, G., Bignamini, D., Kattamis, A., Rachmilewitz, E.A. & Cappellini, M.D. (2006) Prevalence of thromboembolic events among 8,860 patients with thalassemia major and intermedia in the Mediterranean area and Iran. *Thrombosis and Haemostasis*, **96**(4)**,** 488–491.

Tesoriere, L., D'Arpa, D., Maggio, A., Giaccone, V., Pedone, E. & Livrea, M.A. (1998) Oxidation resistance of LDL is correlated with vitamin E status in beta-thalassemia intermedia. *Atherosclerosis*, **137,** 429–435.

Tesoriere, L., D'Arpa, D., Bufera, D., Allegra, M., Renda, D., Maggio, A., Buongiorno, A. & Livrea, M.A. (2001) Oral supplements of vitamin E improve measures of oxidative stress in plasma and reduce oxidative damage to LDL and erythrocytes in beta-thalassemia intermedia patients. *Free Radical Research*, **34,** 529–540.

Tesoriere, L., Allegra, M., Bufera, D., Gentile, C. & Livrea, M.A. (2006) Cytoprotective effects of the antioxidant phytochemical indicaxanthin in beta-thalassemic red blood cells. *Free Radical Research*, **40,** 753–761.

Vento, S., Cainelli, F. & Cesario, F. (2006) Infections and thalassaemia. *The Lancet Infectious Diseases*, **6,** 226–233.

Vichinsky, E.P. (2004) Pulmonary hypertension in sickle cell disease. *New England Journal of Medicine*, **350,** 857–859.

Vogiatzi, M.G., Autio, K.A., Mait, J.E., Schneider, R., Lesser, M. & Giardina, P.J. (2005) Low bone mineral density in adolescents with beta-thalassemia. *Annals of the New York Academy of Sciences*, **1054,** 462–466.

Voskaridou, E., Anagnostopoulos, A., Konstantopoulos, K., Stoupa, E., Spyropoulou, E., Kiamouris, C. & Terpos, E. (2006) Zoledronic acid for the treatment of osteoporosis in patients with beta-thalassemia: results from a single-center, randomized, placebo-controlled trial. *Haematologica*, **91,** 1193–1202.

Weinberg, R.S., Ji, X., Sutton, M., Perrine, S., Galperin, Y., Li, Q., Liebhaber, S.A., Stamatoyannopoulos, G. & Atweh, G.F. (2005) Butyrate increases the efficiency of translation of gamma-globin mRNA. *Blood*, **105,** 1807–1809.

Wilson, S. (2000) Acute leukemia in a patient with sickle cell anemia treated with hydroxyurea. *Annals of Internal Medicine*, **133,** 925–926.

Woo, G.H., Katayama, K., Bak, E.J., Ueno, M., Yamauchi, H., Uetsuka, K., Nakayama, H. & Doi, K. (2004) Effects of prenatal hydroxyurea-treatment on mouse offspring. *Experimental and Toxicologic Pathology*, **56,** 1–7.

Wu, L.C., Sun, C.W., Ryan, T.M., Pawlik, K.M., Ren, J. & Townes, T.M. (2006) Correction of sickle cell disease by homologous recombination in embryonic stem cells. *Blood*, **108,** 1183–1188.