Bone Marrow Transplantation in Thalassemia (Part 1)

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Abstract
During the last two decades conventional therapy has improved the prognosis of thalassemia. However, despite such improvement it still remains a progressive disease with treatment-related complications such as hepatitis, liver fibrosis, and cardiac disease. Bone marrow transplantation (BMT) can prevent or delay progression of the aforementioned complications. The importance of clinical research in the field of BMT was recognized with the award of the 1990 Nobel Prize in Physiology and Medicine to E. Donnall Thomas, one of the pioneers of BMT in humans. George Mathe was a pioneer in the early development of clinical BMT. Mathe et al. were the first to describe graft-versus-host-disease (GVHD) and its treatment, and the graft-versus-leukemia (GVL) effect in human. The first BMT for β-thalassemia major was performed successfully by Thomas et al. in Seattle, in 1981. In the same year another patient with β-thalassemia major underwent BMT in Pesaro, Italy, by Lucarelli et al. Since then, several hundred transplantations have been performed worldwide, the majority of these in Italy. From 1991 through 2007 BMT have been performed on 497 (Tehran=342, Shiraz=155) blood transfusion dependent patients with thalassemia major in Iran, with disease-free survival of 71-77% respectively. Due to high graft failure and GVHD rates, BMT from alternative donors should be restricted to patients who have poor life expectancies because they cannot receive adequate conventional treatment or because of alloimmunization to minor blood antigens. Beginning in the early 1980s, it was shown that umbilical cord blood contained high levels of hematopoietic progenitor cells.


Keywords ● Bone marrow transplantation ● thalassemia ● graft-versus-host-disease

Introduction
More than 20,000 patients are affected by thalassemia major in Iran (19,000 patients are blood transfusion – dependent and the remaining are not). The prognosis of the disease, once invariably fatal within the first decade of life, has improved in recent years.

Abnormalities of hemoglobin are cited as the most prevalent hereditary disorders caused by defects in single gene.1 In areas of the world where falciparum malaria has been endemic, the incidence of these disorders can reach a frequency such that symptomatic, severe disease affects the health care of a significant portion of the population.

Thalassemias are hereditary disorders of hemoglobin synthesis that affect the generation of globin chains. The mechanisms
producing these defects include gene deletions that account for most of \( \alpha \)-thalassemias or point mutations that are the explanation for most \( \beta \)-thalassemias. Over 150 mutations have been reported that affect the transcription or processing of \( \beta \) globin and produce the \( \beta \)-thalassemia phenotype.\(^2\)

Worldwide, an estimated 240,000,000 person are heterozygous for \( \beta \)-thalassemia and an estimated 200,000 infants are born annually with homozygous \( \beta \)-thalassemia.\(^3\) These children are at risk for manifesting the severe phenotype of \( \beta \)-thalassemia major. Community control by prospective heterozygote detection, education, and fetal diagnosis has now been successfully applied in certain European and Mediterranean areas. Within three years of starting these programs, the birth rate of infants with thalassemia major had fallen by 50-80%.

In Iran, screening the prospective couples for \( \beta \)-thalassemia minor was started in Fars province in 1991, and thereafter in 1997 expanded to the entire country. Couples who are both carriers are provided consultation in several sessions. Those decide to marry are referred to prenatal diagnosis labs or family planning units based on their choices.\(^4\)

Although the frequency of abnormalities of \( \alpha \)-globin genes is highest in persons originating from Southeast Asia or West Africa, the incidence of \( \beta \)-thalassemia is highest in populations of the Mediterranean area, Middle East, Indian subcontinent, and Far East. In some regions of Italy and Greece, the frequency of these defective genes may rise to 20-30% of the population.\(^5\) Indeed, 3% of the worldwide population carries a defective gene that contributes to a \( \beta \)-thalassemia phenotype. In certain regions of Greece, Southeast Asia, and the coastal regions of Italy, the birth rate of homozygous \( \beta \)-thalassemia is 1:150 - 1:200.

The carrier rate for \( \beta \)-thalassemia gene in Iran is 4.1 percent of the population, which varies from 0.4 to 9.4 percent in different provinces. The carriers are especially prevalent in the Persian Gulf and Caspian littoral area (7.1-9.4%；figure 1).\(^6\)

During the last two decades, conventional therapy has improved the prognosis of thalassemia. However, despite such improvement, thalassemia still remains a progressive disease with treatment-related complications including hepatitis, liver fibrosis, and cardiac disease progressing with time. Conventional treatment may postpone but not eliminate these complications with the associated morbidity and mortality. In contrast, bone marrow transplantation (BMT) can prevent or delay progression of the aforementioned complications.

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**Figure 1:** The prevalence of thalassemia in Iran -1998. From a report on mass screening of minor \( \beta \) thalassemia and prevention programme in IR. Iran.\(^7\)

**Molecular Bases of Thalassemia**

\( \beta \)-thalassemia most commonly results from single nucleotide substitutions and very rarely through the mechanism of gross gene deletion.

A group of point mutations affect the TATA or CACC boxes and are referred to as promoter mutations. These mutations result in the production of a reduced amount of \( \beta \)-globulin mRNA and thus in the clinical phenotype of \( \beta \)-thalassemia.

A large group of defects are characterized by the non-sense mutation or frame shift mutation which results in the production of in frame stop codon. These mutations produce non-functional \( \beta \)-globulin mRNA and result in clinical phenotype of \( \beta \)-thalassemia.

A third and vast group of mutations, collectively referred to as processing mutations, affect RNA splicing or polyadenilation signal. Those affecting the splicing process include mutations in the coding region which activate a cryptic splice site in the exons, mutations in the splice junction abolishing the normal splicing process, mutations in the consensus sequences at the splice junction reducing the amount of mRNA correctly spliced, and mutations in introns activating a cryptic splice site. Those mutations activating a cryptic splice site in exons and part of those affecting the consensus sequences or activating cryptic splice site in introns result in the production of a certain amount of globin mRNA and therefore express the clinical picture of \( \beta \)-thalassemia.

Mutations at splice junction and part of intron mutations creating a splice site have no globin mRNA production and thus no \( \beta \)-chain synthesis (\( \beta^0 \)-thalassemia).

Polyadenylation signal mutations are associated with the production of some normal globin mRNA and consequently manifest the phenotype of \( \beta^+ \) thalassemia.\(^7\)

The carrier rate for \( \beta \)-thalassemia gene in Fars province, south of Iran is 7.5%,\(^6\) and 10 different mutations were found in a study including...
transcriptional mutants, -88 (C→A), -101 (C→T); RNA processing mutants: splice junction, IVS-I-1 (G→A), IVS-II-1 (G→A); consensus sequence, IVS-I-6 (T→C); other IVS changes, IVS-I-110 (G→A), IVS-II-745 (C→G); nonsense, CD 39 (C→T); frame shift, CD 44 (-C); deletional mutants -25 bp, 3′ IVS-I. The IVS-II-1 (G→A) mutation was the most frequent (31%) followed by the IVS-I-6 (T→C) mutation (15%). Eight mutations were initially described in Mediterranean populations and two were of Kurdish origin. Four of these mutations, both initially described in the Mediterranean region, are reported here for the first time in Iranians. The unexpectedly high numbers of different mutations that account for β-thalassemia in this region of Iran suggest the migration of chromosomes from distant places and genetic admixture. In another study on 67 Iranian individuals, presenting with low mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) levels, normal hemoglobin electrophoresis and iron status, gene deletions and point mutations were tested for the presence of twelve common alpha-thalassemia. Five different mutations (-alpha (3.7), -alpha (4.2), --MED, -(alpha) 20.5, Hb Constant Spring) were identified in a total of 43 cases. The carrier rate for alpha-thalassemia was 4% in Fars province. 

Pathophysiology

The consequence of diminished globin chain synthesis is the precipitation of unpaired globin chains within the developing red blood cell. Precipitation of globin chains results in oxidative damage of the red blood cell membrane, causing increased cellular rigidity, shortened red blood cell survival, and ineffective erythropoiesis (figure 2).

In addition, lack of generation of β- globin chains leads to the use of γ- globin loci to produce fetal hemoglobin (Hb F). The generation of Hb F further increases the erythropoietic drive because this form of hemoglobin has a high affinity for oxygen. Marrow expansion caused by the accelerated erythropoiesis subsequently leads to skeletal abnormalities and increased iron absorption, figure 3).

Medical Management

The cornerstone of medical management for severe forms of β-thalassemia is transfusion of red blood cells. Without transfusion, children with β-thalassemia major die within the first 2 years of life. Simple transfusion to alleviate symptoms related to the anemia, however, do not suppress the increased erythropoietic drive or forestall the development of skeletal or metabolic abnormalities. Therefore, children undergo transfusion to maintain an average hemoglobin level between 9 and 11 g/dl. With the adoption of a hypertransfusion program, persons with clinically severe forms of β-thalassemia experience normal growth and development and are now surviving into third decade of life. The most effective therapeutic protocols include transfusions of leukocyte- filtered red cells every 2-5 weeks designed to maintain mean hemoglobin of 11-12 g/dl. Frequent transfusions have created a second disorder, however- that of iron overload which remains the major cause of death in patients with β-thalassemia major. Economidou reported in 1982 that only 24% of Greek patients with β-thalassemia major and intermedia lived to reach the age of 28 years. The leading cause of death for this population was heart disease secondary to transfusion-related iron overload. Cardiac failure occurred in 63% of patients with β-thalassemia major by the age of 16, and 50% of the patients died within 1 year of its development. These observations led to a more aggressive approach to iron chelation therapy using deferoxamine.

The current recommendation regarding
chelation therapy is to begin deferoxamine (Desferal), 40 mg/kg/day, when the serum ferritin level rises above 1000 ng/ml. The goal of treatment is to maintain the serum ferritin level less than 1000 ng/ml. Because of the short half-life of deferoxamine, it is recommended that it should be administered as continuous subcutaneous infusion over 8-10 hours, 5 nights per week. Nevertheless, iron overload continues to be a frequent complication of thalassemia. Heart disease is the primary cause of death, followed by infection in patients under the age of 15 years. Liver disease occurs, and diabetes mellitus affects 6% of patients over the age of 10 years. Puberty fails to occur in 40% of boys and 25% of girls over 12 years of age. The incidence of side effects form deferoxamine ranges from 2% to 38% with the main adverse effects involving ocular and auditory abnormalities.

Two major problems limit the utility of chelation therapy. Firstly, the estimated annual cost of chelation therapy in 1991 was calculated to be $32,000, with 60% of the total cost related to the expense of deferoxamine per se. In contrast, the estimated cost of allogeneic hematopoietic stem cell transplant (HSCT) at that time was $175,000. Secondly, to achieve the goal of iron chelation during ongoing red blood cell support for β-thalassemia major, compliance with chelation therapy is an important issue. Less than 70% of older patients with β-thalassemia major have been reported to be compliant with deferoxamine-based chelation therapy.

It was initially hoped that oral chelation agents such as deferiprone (Ferripro/L1) would supplant the need for daily parenteral administration of deferoxamine. A 1998 study, however, suggested that deferiprone is not as efficacious as deferoxamine in reducing total body iron and is associated with an increased risk of hepatic fibrosis. Deferiprone is an orally administered iron chelator that has been approved for use as second-line chelation therapy in Europe since 1999. Initial concerns regarding an increased risk of hepatic fibrosis have proved unfounded, and there are now considerable clinical trial data to suggest that deferiprone is superior in removing myocardial iron compared with deferoxamine; this position has been strengthened with data showing improved outcomes in patients on deferiprone. Conversely, deferiprone may be less effective in removing hepatic iron than deferoxamine. Because of the relative merits of the two drugs, the issue of combined therapy has been proposed (deferiprone: 75 mg/kg/day, divided into three doses plus deferoxamine 2 nights per week).

In comparison with the standard chelation monotherapy of deferoxamine, combination treatment with additional deferiprone reduced myocardial iron and improved the ejection fraction and endothelial function in patients with thalassemia major with mild to moderate cardiac iron loading. Myocardial iron loading was assessed by the use of myocardial T2* cardiovascular magnetic resonance.

The most serious adverse event associated with deferiprone is agranulocytosis. Though it occurs only in approximately 1% of patients, all patients on deferiprone need to have their blood counts monitored weekly to ensure that their absolute neutrophil count (ANC) does not drop to potentially dangerous levels. The etiology of agranulocytosis is considered idiosyncratic. If agranulocytosis occurs, deferiprone therapy should be discontinued immediately and granulocyte colony stimulating factor (G-CSF) can be helpful. Neutropenia, a less serious side effect, occurs in about 4-6% of the patients.

Despite the availability of chelation therapy for the last 40 years, iron overload remains a clinically significant issue for patients with thalassemia. Deferasirox (Exjade) is a new once-daily oral iron chelator (20 mg/kg) that exhibits the key characteristics wanted in a new chelator. These include: i) oral bio-availability, ii) a high affinity to and specificity for Fe3+, iii) a prolonged biological half-life, and iv) low predictable toxicity.

Bone marrow transplantation (BMT) has been introduced as an alternative approach to the treatment of thalassemia after animal studies had shown the feasibility of curing genetically determined hemolytic anemias by marrow grafting, and good results had been obtained in patients with leukemia and aplastic anemia.

History of Bone Marrow Transplantation

The importance of clinical research in the field of BMT was recognized with the award of the 1990 Nobel Prize in Physiology and Medicine to E. Donnall Thomas, one of the pioneers of BMT in humans. Bone marrow was first used to treat human diseases in 1891 by Brown-Sequard, and this was reported by Quine in 1896. One dram of an aromatic glyceride of red marrow was given orally after meals for the treatment of leukemia. Subsequently, attempts were made to treat pernicious anemia using a glycerol extract of animal bone marrow administered orally, and in 1899 intramedullary injections of marrow were used to treat aplastic anemia with some success. However, any positive effects seen after such therapy were refuted by Billings (1894) and Hamilton (1895), who probably correctly, attributed...
them to the mineral and iron content of the material. Saline extracts of red bone marrow and spleen were also tried as hemopoietic stimulants and some successes were observed where other treatments had failed. In 1937 Schretzenmayr administered intramuscular injections of freshly aspirated autologous or allogeneic bone marrow to patients suffering from parasitic infections, again with some success.30

The first intravenous infusion of bone marrow was carried out by Osgood.31 Jacobson et al. (1951),32 showed that mice could recover from lethal irradiation if the hemopoietic areas in their femurs were shielded, having previously observed that marrow aplasia in irradiated mice could be reversed by shielding the spleen (1949).33 Subsequently, mice given potentially lethal doses of radiation were protected by marrow infusion, and in 1952 Lorenz showed that recovery was due to the cells in transplanted marrow.34

Immediately after the World War II, the hematological effects observed following irradiation in atomic bomb survivors of Hiroshima and Nagasaki stimulated research into the potential of bone marrow to confer radioprotection in experimental animals. The idea of marrow transplantation was rapidly taken up by experimental clinicians who observed its potential for correcting bone marrow failure syndromes, and as a means of protecting patients against the myeloablative effects of radiation and chemotherapy.35

The problems confronting clinicians in the early years of bone marrow transplantation were numerous. Autologous marrow transplantation necessitated the development of reliable systems for cryopreserving marrow (Pegg, 1966),36 and allogeneic BMT was beset by the immunological problems of graft rejection, and the poorly understood phenomenon of ‘secondary disease’ (Barnes et al., 1962).37 Until the human leukocyte antigen (HLA) system was recognized and HLA typing developed, the only compatibility test available was the mixed lymphocyte culture (MLC), developed by Friedman in 1961.38 Without proper matching, donor selection was a random affair, and because of the high risk of serious incompatibility, only occasional transplants were successful. There were, however, seven reported cases of correction of aplastic anemia by syngeneic transplant, and at least four of these may well have represented successful correction of hematopoiesis by BMT.39,40 However, the novelty of BMT as a therapeutic modality in the minds of most clinicians was such that these reports were treated with skepticism.

Until mid 60s, allogeneic hemopoietic stem cell infusions were not established as a practical procedure in clinical medicine. Out of 417 reported cases, only three prolonged allografts were documented and no more than 10% of the patients experienced clinical improvement attributable to the marrow infusion (Pegg, 1966).36

George Mathe’ was a pioneer in the early development of clinical BMT. He was the first to propose the need for a large radiation dose to eliminate recipient malignancy, the use of substantial amounts of donor marrow to ensure engraftment, and the application of sterile nursing techniques. In 1958, six physicists were accidentally exposed to large doses of mixed X and neutron irradiation at Vinca in Yugoslavia.41 The most severely irradiated died, however, of the remaining five, four were judged to have received a radiation dose of 600-1000 rads. They were treated with allogeneic bone marrow infusions, and red cell antigen studies demonstrated that successful but temporary engraftment ensued. It is also of interest that donor red cell output paralleled the amount of marrow initially infused. Presumably, autologous hemopoietic recovery occurred eventually, but the allogeneic bone marrow served to protect the patients until recovery occurred.

Mathe’ et al. (1963) were the first to describe graft-versus-host-disease (GVHD) in human.42 A leukemic patient, conditioned with methyl-nitro-imidazole, mercaptopurine, and total body irradiation (TBI) followed by allogeneic bone marrow infusion from six donors, developed desquamating dermatitis, diarrhea and weight loss. The patient was treated with steroids and antibiotics, and recovered. Mathe’ suggested at that time that the occurrence of secondary disease might favor elimination of leukemia cells, and may have helped toward achieving remission in this case.43 These early proposals have now been substantiated, and are termed the graft-versus-leukemia (GVL) effect.44 There is a smaller incidence of leukemia relapse in patients surviving GVHD compared with the patients experiencing no GVHD.

McFarland in 1961 treated 20 patients with aplastic anemia with no marrow ablation.45 He used a cell dose of 0.7-40×10^9 nucleated cells, and seven patients showed improvement, with five recovering completely. Prior to this, very small quantities of bone marrow had been used, which almost certainly contained insufficient stem cells to affect engraftment. In general, however, successful ‘take’ of the infused marrow did not parallel an ultimately successful clinical outcome. In many cases there was graft failure after initial documented take, and it was rare for true chimerism to result. It was therefore realized that some form of 'condition-
ing prior to allogeneic bone marrow infusion was necessary in order to suppress host reaction to infused bone marrow, and in the cases of malignant disease to eradicate evidence of the disease prior to BMT.

Much of the subsequent work concerning the development of BMT was carried out by E. Donnall Thomas (1957). He initially concerned himself with autologous BMT and systematically examined the various components of the BMT procedure. He used the dog as an experimental model to develop effective TBI schedules and introduced methotrexate as a means of preventing GVHD. These technical advances, the characterization of the HLA system by Van Rood et al., (1958), Payne (1964), and Daussel (1965), and the miniaturization of the HLA assay by Terasaki and Mc Lelland in 1964, created a new era in BMT where transplants were carried out between HLA- matched sibling donor-recipient pairs. Pioneering work in severe combined immunodeficiency disease (SCID) by the Leiden group demonstrated the ability of BMT to cure this otherwise fatal disorder. In Seattle, led by Donall Thomas, BMT was carried out with increasing success in aplastic anemia and leukemia. The Seattle group maintained a lead in the clinical application of BMT which persists to this day.

**Tissue Typing**

*The Human Leukocyte Antigen (HLA) System*

HLA expression is not confined to leukocytes. Most tissues including erythrocytes and platelets express class I antigens. However, class II molecules are expressed by only a limited range of cells including activated T lymphocytes, B cells, macrophages, dendritic cells, and Kupffer cells. The primary function of the HLA system is to react with foreign antigens in the context of HLA identity rather than disparity, although the ability to recognize allogeneic HLA molecules even if never previously exposed to them is well preserved. So biological functions of the major histocompatibility complex (MHC) are differentiation between self and non-self, key-molecules for the initiation of the specific immune- response, ligand for natural killer cells (NK- cells), and maturation of T- lymphocytes. This is now known to be present in all mammalian species. The genes of the human MHC are located on chromosome 6 (figures 4, 5).

The class I of MHC interacts closely with the CD8 antigen on T cells. Class II molecules consist of α and β-chain. They are transmembrane molecules and interact closely with the CD4 antigen of the T-helper cells. In transplantation, HLA- DR is the most important of the class II molecules.

There are also several 'minor' loci outside the MHC region that encode molecules thought to be important and are strongly implicated in the pathogenesis of GVHD and in histocompatibility recognition.

The three loci of major importance in BMT are the HLA-A, B and DR B1. Their closely linked, multiple alleles lead to four possible haplotype combinations within a family. There is, therefore, one in four chance of a sibling donor being a match for a patient. Tissue typing identifies the phenotype of patients and potential donors. Serological test are used for A, B, and DR typing and a mixed lymphocyte reaction (MLR) is used to confirm DR identity. Molecular biology techniques are used to give accurate HLA typing including antigen-level (low resolution), allele group-level (intermediate resolution), and allele-level (high resolution; figure 6).

Up to now there are 506 A, 851 B, 275 C, 476 DRB1, 81 DQB1 and 126 DPB1 recognized alleles. HLA molecular typing is shown by asterisk* (figure 7).
Bone marrow transplantation in thalassemia (part 1)

The first large series of BMT for patients with β-thalassemia was reported in Pesaro in 1984.56 Thirteen patients, seven of whom underwent transplantation late in the course of disease, received HLA-matched allogeneic marrow after a variety of myeloablative conditioning regimens, including TBI with or without busulfan, and with or without cyclophosphamide. Among the six deaths, five were thought to be related to the conditioning regimen. Five of the remaining seven patients experienced reconstitution of their β-thalassemia, whereas only two patients were long-term survivors and were considered to be cured. From this early experience, the Pesaro group revised both their conditioning regimen and their target population by eliminating TBI and limiting transplantation to younger children.

The following year, the Pesaro group reported their findings of allogeneic BMT limited to patients who were less than 8 years old and who received busulfan and cyclophosphamide as conditioning regimen.57 Of six patients receiving busulfan (16 mg/kg) plus cyclophosphamide (200 mg/kg), three died due to problems related to transplantation. The next 24 patients received the same dose of cyclophosphamide but a lower amount (14 mg/kg) of busulfan. Only one subsequent death was attributed to the conditioning regimen. The OS rate for the 26 patients was 86%, with a DFS rate of 78%. AGVHD developed in 23% of the patients despite prophylaxis with methotrexate with or without cyclosporine. Graft rejection was reported 12% for this group. By limiting the protocol to younger children and using cyclophosphamide and lower dose of busulfan as the myeloablative regimen, in lieu of TBI the researchers reduced the incidence of graft rejection and transplant-related mortality to an acceptable level.

A subsequent trial in Pesaro involved 40 older children (8-15 years) with β-thalassemia using busulfan (14 mg/kg) plus cyclophosphamide (200 mg/kg) as the conditioning regimen and was also successful.58 Overall survival and DFS rates in this study were 75% and 69%, respectively. Graft rejection and mortality rates remained at about 10% however; a higher incidence of AGVHD was noted (34%). Thus, age as a factor for transplantation was increased to those children younger than 16 years.

In 1990, a retrospective analysis of the first 222 patients less than 16 years of age who underwent allogeneic BMT in Pesaro was reported.59 Among the entire group of patients, OS and DFS rates were 82% and 75% respectively. Univariate analysis of factors associated with a poor outcome, however, demonstrated that three conditions adversely affected

Hematopoietic Stem Cell Transplantation in Thalassemia

The first BMT for β-thalassemia major was performed successfully in a 16-month-old Italian boy whose parents refused transfusions for religious reasons, from his HLA-identical sister, by Thomas et al. in Seattle, in 1981.53 He is now 27 years old and in excellent general condition.54 In the same year, a 14-year-old Iranian patient (personal communication, 1998) with β-thalassemia major underwent BMT in Pesaro, Italy, by Lucarelli et al. However, this patient experienced rejection of the graft and reconstitution of the thalassemia phenotype. Since then, several hundred transplants have been performed worldwide, the majority of them in Italy. The world experience had been reviewed (Borgna-Pignatti, 1992).55 The Patients' ages at BMT have been between 6 months and 24 years, and the number of transfusions received has ranged from none to several hundred. A few patients underwent BMT for thalassemia intermedia and a few others were affected by Hb E/β-thalassemia or Hb S/β-thalassemia. Overall survival (OS) among patients with a follow up of at least three months was 80%, ranging in the four largest series from 82% to 95%. Disease-free survival (DFS) was 67% (67-90%).

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Figure 6: HLA resolution degree of typing. Extracted from Mytilineos J: The HLA- System: Genetics, Function and Typing Methods. Lecture held at the Advanced ICAS Training Course on Blood Stem Cell Transplantation, 17 April 2007 at the University of Ulm.52

Figure 7: The number of HLA alleles. Extracted from Mytilineos J: The HLA- System: Genetics, Function and Typing Methods. Lecture held at the Advanced ICAS Training Course on Blood Stem Cell Transplantation, 17 April 2007 at the University of Ulm.52

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In 1990, a retrospective analysis of the first 222 patients less than 16 years of age who underwent allogeneic BMT in Pesaro was reported.59 Among the entire group of patients, OS and DFS rates were 82% and 75% respectively. Univariate analysis of factors associated with a poor outcome, however, demonstrated that three conditions adversely affected
outcome: 1) the presence of hepatomegaly as defined by enlargement of the liver greater than 2 cm below the costal margin, 2) the presence of liver fibrosis in the pretransplantation liver biopsy, and 3) the quality of chelating therapy received before BMT. The latter was considered adequate when deferoxamine therapy was initiated within 18 months after the first transfusion and was administered subcutaneously for 8-10 hours for at least 5 days weekly. Chelation therapy was considered inadequate if there was any deviation from these requirements. Patients without any of these risk factors (i.e., class I) had an OS/DFS rate of 94%: 94%. In addition, no patients experienced graft rejection in this group. In patients with one or two risk factors (i.e., class II), the OS and DFS rates were 80% and 77% respectively, with 9% of patients experiencing graft rejection. Finally, among patients with three risk factors (i.e., class III), OS, DFS, and rejection rates were 61%, 53%, and 16% respectively. All patients with class I, but only 33% of patients with class II and 8% with class III risk factors, were determined to have received adequate chelation therapy. Thus, the degree of end-organ damage secondary to iron overload was found to be a major contributing factor to outcome.60

Although results in children less than 16 years of age continued to improve, the initial experience in BMT for young adults had been less successful.57 In 1992, the Pesaro group based an adjustment in the concentration of cyclophosphamide from the standard 200 mg/kg to 120-160 mg/kg on the hypothesis that the increased morbidity and mortality observed in older patients primarily was due to cardiac and liver toxicity.61 The OS, DFS, and rejection rates among 26 adult patients (age range 17-26 years) in this study were 85%, 80% and 5% respectively. Mortality related to BMT remained low (3 of 26, or 12%). The benefit of the adjusted dose of cyclophosphamide was also shown in a larger study of 214 patients with class III risk factors.62 The OS and no rejection-related mortality rates for the entire group were 62% and 32% respectively. Among 95 patients in class III who received the adjusted dose of cyclophosphamide, however, the OS and no rejection mortality rates were 74% and 24% respectively. The rejection rate for both groups remained higher than patients in class I or II (22% or 35% v <10%, respectively). Interestingly, an analysis of risk factors associated with rejection demonstrated that patients in class III who received fewer than 100 transfusions had a 53% incidence of rejection, whereas those who received more than 100 transfusions had a 24% chance of rejection. Thus, in older patients, reducing the dose of cyclophosphamide decreased the mortality in patients with greater transfusion-related (and therefore iron-associated) organ dysfunction. Nonetheless, increased exposure to transfusions appears to diminish the risk of rejection.60

Analyses of BMT in thalassemia performed in many other centers around the world have been reported at the Third International Symposium on BMT in Thalassemia held in Pesaro in September 1996.63 The DFS have been 53-88% (table 1).

### Table 1: Reported transplants for thalassemia and Kaplan and Meier probabilities of survival and disease-free survival in various international transplant centers.

<table>
<thead>
<tr>
<th>Center</th>
<th>Patients</th>
<th>Survival (%)</th>
<th>Disease-free survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pescara</td>
<td>102</td>
<td>91</td>
<td>87</td>
</tr>
<tr>
<td>Cagliari 1</td>
<td>25</td>
<td>80</td>
<td>72</td>
</tr>
<tr>
<td>Cagliari 2</td>
<td>37</td>
<td>88</td>
<td>88</td>
</tr>
<tr>
<td>U.S.A</td>
<td>68</td>
<td>87</td>
<td>70</td>
</tr>
<tr>
<td>U.K</td>
<td>50</td>
<td>90</td>
<td>76</td>
</tr>
<tr>
<td>Tehran</td>
<td>60</td>
<td>83</td>
<td>73</td>
</tr>
<tr>
<td>Vellore</td>
<td>50</td>
<td>76</td>
<td>68</td>
</tr>
<tr>
<td>Malaysia</td>
<td>28</td>
<td>86</td>
<td>75</td>
</tr>
<tr>
<td>Hong Kong</td>
<td>25</td>
<td>86</td>
<td>63</td>
</tr>
<tr>
<td>Bangkok</td>
<td>21</td>
<td>76</td>
<td>53</td>
</tr>
</tbody>
</table>


At this symposium, it was reported that more than 1000 patients have received BMT in centers in Europe, North America, and Asia, with an OS rate of about 80%.64 Among the surviving patients, nearly 90% have been cured.

The published summary from the group in Pesaro, involving 826 patients continues to show the efficacy of BMT in β-thalassemia (table 2).60 From this summary it is apparent that both OS and DFS diminish with increasing age or class, probably reflecting organ damage mediated by excess iron. Therefore, Pesaro group recommends that children with an HLA-matched family donor undergo transplantation while they are in class I. Delaying the BMT until the patient is in class II or higher reduces the success rate and jeopardizes the reversibility of liver and cardiac damage.63

The report from Guido Lucarelli and his group in Pesaro during the past 20 years, which was reported at the 30th European BMT meeting held in Barcelona, Spain in March 2004, is that from December 17, 1981 through January 21, 2003 one thousand and three (1003) consecutive patients aged from 1 to 35 years have been transplanted in Pesaro (950 from an HLA identical related donor, 42 from partially matched
related donors and 11 from a matched unrelated donors). Overall the >20 years Kaplan Meier probability of survival was 68%,\(^6^5\) (figure 8).

Patients in class I (low risk) and class 2 (intermediate risk) were transplanted after conditioning with the association of busulfan (14 mg/kg) and cyclophosphamide (200 mg/kg). This conditioning regimen is unmodified since 1985. Patients in class 3 (advanced disease) have been transplanted using protocols that include lower dosages of cyclophosphamide (120-160 mg/kg). Transplantation in class 3 patients using a reduced dose of cyclophosphamide was characterized by a 30% risk of thalassemia recurrence.

In April 1997 a new preparative regime was elaborated with the aim to increase sustained engraftment rate and decrease transplant related mortality in class 3 patients. This new preparative regimen is characterized by the addition of azathioprine, hydroxyurea, and fludarabine to the ablative association of busulfan 14 mg/kg and cyclophosphamide 160 mg/kg. Results obtained in 33 patients younger than 17 years in class 3 are consistent with 90% thalassemia-free survival.

Adult patients have been transplanted according to risk class and protocol in use. Of 109 consecutive adult patients submitted to allogeneic HLA identical sibling transplantation, overall survival was 66%, thalassemia free survival 62%, transplant related mortality 36% and thalassemia recurrence 4%.

The reported transplants for thalassemia from some international transplants centers are shown in table 3, the DFS is 50-100%.

From 1991 through 2007, BMT have been performed on 497 (Tehran=342, Shiraz=155) blood transfusion dependent patients with thalassemia major in Iran, with DFS of 71-77% respectively.

Seventy patients with beta thalassemia major underwent bone marrow transplantation during 1991-1997 in Shariati hospital in Tehran, Iran. The survival and rejection curves leveled off at 8 and 18 months after transplantation at 82.6% and 11.4%, respectively. Pre-transplant clinical features (age, serum ferritin, portal fibrosis, hepatomegaly and quality of chelation therapy) were examined for their effects on survival and recurrence of thalassemia in this group of patients who were less than 16 years old. Increasing age, presence of portal fibrosis and increasing serum ferritin were significantly associated with reduced probability of survival. This study also showed the benefit of transplanting more than 5.5\(\times\)10^8/kg mononuclear cells in the patients with no increase in complications.\(^6^8\)

One hundred and twelve patients with the diagnosis of beta thalassemia major underwent allogeneic marrow transplantation from HLA identical or one antigen-mismatched related donors during June 1993 to January 2003 in


\(^{16}\) EFS, event-free survival; Bu, busulfan; Cy, cyclophosphamide; CSA, cyclosporine; MTX, methotrexate
Shiraz, Iran. The mean age of the patients was 9.5 years with the range of 2 to 20 years. The distribution of the patients according to the Lucarelli classification was: 27 patients in class I, 38 patients in class II, and 47 patients in class III. Eighty seven of the 112 patients (77.6%) are living with full engraftment at a median follow up of 6 years (range 2 to 119 months).

In another report from Shiraz, Iran, on 90 transfusion-dependent patients with thalassemia major who were transplanted up to December 2001, the donors were HLA-identical, MLC-non reactive siblings (n=74) or parents (n=6); HLA-identical MLC-reactive siblings (n=5) or parents (n=1); and one HLA antigen mismatched siblings (n=4). The induction regimen in 11 patients was oral busulfan (BU) (14 mg/kg) and IV cyclophosphamide (CY; 200 mg/kg); in 15 patients it was BU (15 mg/kg), and cyclophosphamide (CY; 200 mg/kg); in 47 patients, BU (15 mg/kg), CY (200 mg/kg), and short course of anti-thymocyte globulin (ATG; horse; 40 mg/kg including 10 mg/kg on days -2, -1 , +1, +2); and in 15 patients, BU (15 mg/kg), CY (200 mg/kg), and ATG (60 to 100 mg/kg, 10 mg/kg at 3 to 5 days before and after BMT). Graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporine and methyl prednisolone. The group who received BU (14 mg/kg) and CY (200 mg/kg) as compared with the group receiving BU (15 mg/kg) and CY (200 mg/kg), were younger with lower risk; median age 7 versus 10 years, and 46% versus 7% in Lucarelli's risk group class I (the best prognostic group), respectively. These patients showed a lower disease-free survival (DFS), namely 64% versus 73%, with a follow up of 2 to 10.5 years. Their standard protocol for BU has been 15 mg/kg from 1995. The group who received "short" ATG

### Table 3: Reported transplants for thalassemia in some international transplant centers.

<table>
<thead>
<tr>
<th>Center /Reference</th>
<th>Conditioning regimen (mg/kg)</th>
<th>Number of patients</th>
<th>Survival %</th>
<th>DFS %</th>
</tr>
</thead>
<tbody>
<tr>
<td>New York, USA (1998),66</td>
<td>TBI: 720 cGY, CY:120 or BU:14, CY:200</td>
<td>13</td>
<td>92</td>
<td>85</td>
</tr>
<tr>
<td>California, USA (1998),67</td>
<td>BU:1200 cGY, CY:120</td>
<td>6</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>Tehran, Iran (1998),68</td>
<td>BU:14, CY:200</td>
<td>8</td>
<td>100</td>
<td>71</td>
</tr>
<tr>
<td>India (2001),69</td>
<td>BU:16, CY:200 ±ATG</td>
<td>106</td>
<td>74</td>
<td>70</td>
</tr>
<tr>
<td>Turkey (2001),70</td>
<td>BU:16, CY:200 or BU:14, CY:160 + ATG (PSCT)</td>
<td>15</td>
<td>87</td>
<td>87</td>
</tr>
<tr>
<td>Hong Kong (2002),71</td>
<td>BU:16, CY:150-200 ±ATG</td>
<td>44</td>
<td>86</td>
<td>82</td>
</tr>
<tr>
<td>China (2003),72</td>
<td>BU:16, CY:200, ±ATG</td>
<td>15</td>
<td>100</td>
<td>60</td>
</tr>
<tr>
<td>Dutch (2003),73</td>
<td>BU:16, CY:200, ±ATG +Melphalan:140 mg/m²</td>
<td>11</td>
<td>90</td>
<td>64</td>
</tr>
<tr>
<td>United Kingdom (2003),74</td>
<td>BU:16, CY:200, ±ATG +Melphalan:140 mg/m²</td>
<td>10</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>Pesaro, Italy (2004),65</td>
<td>BU:14, CY:120-200 ±ATG:100</td>
<td>1003</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>Rome, Italy (2004),75</td>
<td>BU:14, CY:160 +Azathioprine:102 Hydroxyurea:1020 Fludarabine:100 mg/m2 Hypertransfusions Desferrioxamine:1360</td>
<td>33</td>
<td>93</td>
<td>85</td>
</tr>
<tr>
<td>Shiraz, Iran (2004),76</td>
<td>BU:14-15, CY:200 ±ATG:40</td>
<td>112</td>
<td>88</td>
<td>77</td>
</tr>
<tr>
<td>Shiraz, Iran (2005),77</td>
<td>BU:14-15, CY:200 ±ATG:40-100</td>
<td>88</td>
<td>83</td>
<td>72</td>
</tr>
<tr>
<td>Saudi Arabia (2006),78</td>
<td>BU, CY ± ATG</td>
<td>50</td>
<td>92</td>
<td>77</td>
</tr>
<tr>
<td>Taiwan (2006),79</td>
<td>BU:14, CY:200 or BU:14, CY:160 ±Hydroxyurea, Azathioprine, Fludarabine</td>
<td>26</td>
<td>87</td>
<td>65</td>
</tr>
<tr>
<td>Pakistan (2007),80</td>
<td>BU:14, CY:200</td>
<td>48</td>
<td>79</td>
<td>75</td>
</tr>
</tbody>
</table>

DFS: disease-free survival, PSCT: peripheral stem cell transplant, TBI: total body irradiation, BU: Busulfan, CY: Cyclophosphamide, ATG: Anti-thymocyte globulin.

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D. Zakerinia

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The data are shown on tables 4 and 5.

especially when an HLA disparity was present. GVHD was reduced in patients with thalassemia, the incidence of grade IV acute and chronic "short" ATG course to the conditioning regimen, 15 mg/kg for its myeloablative properties. By adding at a dose of 15 mg/kg was more effective than 14 mg/kg versus those received 60 to 100 mg/kg, patients who received ATG with dose of 40 mg/kg versus 61 to 65 days. Second bone marrow infusions was needed in 6% and 20% of patients who received ATG with dose of 40 mg/kg and chronic GVHD, respectively. There was no significant difference in reduction of platelets and white blood cell counts or engraftment days and the number of packed red blood cell transfusions among the groups. The median hospital stay was longer for the group with BU (15 mg/kg), CY (200 mg/kg) namely 81 versus 61 to 65 days. Second bone marrow infusions was needed in 6% and 20% of patients who received ATG with dose of 40 mg/kg versus those received 60 to 100 mg/kg, respectively (1 to 2 months post-BMT). Busulfan at a dose of 15 mg/kg was more effective than 14 mg/kg for its myeloablative properties. By adding "short" ATG course to the conditioning regimen, the incidence of grade IV acute and chronic GVHD was reduced in patients with thalassemia, especially when an HLA disparity was present. The data are shown on tables 4 and 5.77

In Pesaro, Italy, 29 patients with thalassemia with a median age of 6 years (range 1.1-33 years) were given a BMT from an alternative donor.81 Six of the 29 donors were HLA-phenotypically identical and two were mismatched relatives (four cousins, two aunts, one uncle, and one grandmother), 13 were mismatched siblings and eight were mismatched parents. Six patients received no antigen (relatives), 15 patients one antigen, five patients two antigen and three patients three antigen disparate grafts. Twenty-three patients were in class 2 or class 3, whereas six patients were in class 1. The distribution of patients according to geographical regions was as follows: Italy 11 patients, Middle East six patients, India three patients, Iran three patients, other European countries three patients, Argentina, Azerbaijan and Pakistan one patient from each country. Thirteen patients were given BU/CY, nine patients BU/CY plus anti-lymphocyte globulin (ALG), six patients BU/CY plus TBI or total lymphoid irradiation (TLI) and one patient BU/CY with prior cytoreductive-immunosuppressive treatment as conditioning. For GVHD prophylaxis, four patients received methotrexate (MTX), 22 Cyclosporine (CsA) plus MTX plus methylprednisolone (MP) and three patients CsA plus MP. Thirteen of the 29 patients (44.8%) had sustained engraftment. The probability of graft failure or rejection was 55%. There was no significant difference between antigen disparities and graft failure. The incidence of grade II-IV acute GVHD was 47.3% and incidence of chronic GVHD was 37.5%. The incidence of acute GVHD was higher in patients receiving one or two antigen disparate in the GVHD direction grafts (v no antigen). The probability of overall and event- free survival (EFS) was 65% and 21%, respectively, with median follow-up of 7.5 years (range 0.6-17 years) for surviving patients. The degree of HLA disparity between patient and donor did not have a significant effect on survival. The incidence of non-hematologic toxicity was low. Transplant-related mortality (TRM) was 34%. GVHD (acute or chronic) was a major cause of death (50%) followed by infections (30%). Authors concluded that at present, due to high graft failure and GVHD rates, BMT from alternative donors should be restricted to patients who had poor life expectancies because they could not receive adequate conventional treatment or because of alloimmunization to minor blood antigens.81

Human Immune Deficiency Virus and Bone Marrow Transplantation

Before donated blood was systematically screened, 57 out of 3633 (1.5%) European patients had become HIV-positive.82 With screening, the risk of receiving an infected unit of blood appears to remain in the order of 1:50 000.83 Two HIV-positive patients have undergone BMT to date, but in both, the procedure was soon followed by death.

Hepatitis C Virus Infection and Bone Marrow Transplant in Thalassemia

In Pesaro, Italy, 98 patients with homozygous beta-thalassemia who had undergone allogeneic BMT between May 1990 and March 1992 were tested for hepatitis C antibodies (anti- HCV) before and after BMT. Anti- HCV positivity was detected in 50 of the 98 patients (51%) before BMT. Of the 46 evaluated seropositive patients, four had transient and five had persistent negativity for HCV antibodies after BMT. A strong correlation was found between biochemical and histological evidence of liver damage and anti-HCV positive status in multi-transfused patients. In this study, HCV hepatitis did not influence the outcome of BMT.84
Table 4: Transplant and post-transplant data in patients with thalassemia major based on the different conditioning regimens in Shiraz, Iran (1993-2003).

<table>
<thead>
<tr>
<th>Groups</th>
<th>A₁</th>
<th>A₂</th>
<th>B₁</th>
<th>B₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>BU: Conditioning regimens (mg/kg)</td>
<td>14</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>CY: Bone marrow volume, range (median), ml</td>
<td>200-680 (400)</td>
<td>350-1250 (800)</td>
<td>330-1200 (650)</td>
<td>400-1240 (800)</td>
</tr>
<tr>
<td>ATG: Total nucleated cells of donor marrow / kg body weight of recipient, range (median)</td>
<td>4-10 (5) ×10⁸</td>
<td>1.8-8.7 (3.3) ×10⁸</td>
<td>2.7-8 (4.4) ×10⁸</td>
<td>2.3-7 (3.5) ×10⁸</td>
</tr>
<tr>
<td>Day of plts drop to &lt;100,000/mm³</td>
<td>0-+8 (+4)</td>
<td>+1 - +6 (+4)</td>
<td>-3 - +6 (+4)</td>
<td>-1 - +8 (+3)</td>
</tr>
<tr>
<td>Day of WBC drop to &lt;1000/mm³</td>
<td>0-7 (+3)</td>
<td>-2 - +6 (+2)</td>
<td>-2 - +5 (+2)</td>
<td>0-+6 (+1)</td>
</tr>
<tr>
<td>Day of plts engraftment (&gt;30,000/mm³), range (median)</td>
<td>+16-49 (+23)</td>
<td>+13 - +74 (+23)</td>
<td>+5 - +125 (+20)</td>
<td>+9 - +64 (+25)</td>
</tr>
<tr>
<td>Day of PMN engraftment (&gt;500/mm³), range (medium)</td>
<td>+12 - +33 (+15)</td>
<td>+11 - +28 (+14)</td>
<td>+10 - +92 (+19)</td>
<td>+11 - +59 (+15)</td>
</tr>
<tr>
<td>Second BM infusion, n(%), (days, post BMT)</td>
<td>1 (9)</td>
<td>-</td>
<td>5 (36)</td>
<td>3 (20)</td>
</tr>
<tr>
<td>No of plts concentrate transfusion, range (median)</td>
<td>3-26 (6)</td>
<td>5-17 (8)</td>
<td>2-34 (10)</td>
<td>2-26 (11)</td>
</tr>
<tr>
<td>No of RBC transfusion, Range (median)</td>
<td>2-9 (3)</td>
<td>1-10 (5)</td>
<td>0-13 (4)</td>
<td>0-13 (5)</td>
</tr>
<tr>
<td>Hospital stay, range (median), days</td>
<td>52-130 (65)</td>
<td>50-133 (81)</td>
<td>44-175 (61)</td>
<td>52-119 (65)</td>
</tr>
<tr>
<td>Acute GVHD, n (%), Grade I</td>
<td>6 (55)</td>
<td>2 (18)</td>
<td>1 (9)</td>
<td>1 (9)</td>
</tr>
<tr>
<td>Chronic GVHD</td>
<td>2 (18)</td>
<td>3 (27)</td>
<td>1 (7)</td>
<td>1 (7)</td>
</tr>
<tr>
<td>Stable, n</td>
<td>3 (27)</td>
<td>4 (27)</td>
<td>3 (6)</td>
<td>3 (6)</td>
</tr>
<tr>
<td>Death, n (%), day, Post BMT</td>
<td>2 (18)</td>
<td>22,365</td>
<td>3 (20)</td>
<td>15 (17)*</td>
</tr>
<tr>
<td>Mixed chimerism, n(%), day, post BMT</td>
<td>3 (27)</td>
<td>1 (7)</td>
<td>3 (6)</td>
<td>1 (7)</td>
</tr>
<tr>
<td>Stable, n</td>
<td>2 (18)</td>
<td>3 (20)</td>
<td>4 (9)</td>
<td>1 (7)</td>
</tr>
<tr>
<td>Recurrence of Thalassemia n (%), day, post BMT</td>
<td>190</td>
<td>90,150,600</td>
<td>60,90,90,90</td>
<td>120</td>
</tr>
<tr>
<td>Full donor chimerism, n(%)</td>
<td>5 (54)</td>
<td>10 (67)</td>
<td>31 (66)</td>
<td>10 (67)</td>
</tr>
<tr>
<td>Disease-free survival, n (%)</td>
<td>7 (64)</td>
<td>11 (73)</td>
<td>34 (72)</td>
<td>11 (73)</td>
</tr>
<tr>
<td>Overall survival n (%)</td>
<td>9 (82)</td>
<td>14 (93)</td>
<td>38 (81)</td>
<td>12 (80)</td>
</tr>
</tbody>
</table>


Table 5: Outcome of bone marrow transplant in patients with thalassemia major based on the different conditioning regimens in Shiraz, Iran (1993-2003)

<table>
<thead>
<tr>
<th>Group</th>
<th>A₁ (n=11)</th>
<th>A₂ (n=15)</th>
<th>B₁ (n=47)</th>
<th>B₂ (n=15)</th>
<th>Total (n=88)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follow up, range (median), year</td>
<td>9-10.5</td>
<td>2-9</td>
<td>2-9</td>
<td>4-7</td>
<td>2-10.5</td>
</tr>
<tr>
<td>Death, n (%), day, Post BMT</td>
<td>2 (18)</td>
<td>1 (7)</td>
<td>9 (19)</td>
<td>3 (20)</td>
<td>15 (17)*</td>
</tr>
<tr>
<td>Mixed chimerism, n(%), day, post BMT</td>
<td>3 (27)</td>
<td>1 (7)</td>
<td>3 (6)</td>
<td>1 (7)</td>
<td>8 (9)</td>
</tr>
<tr>
<td>Stable, n</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>7 (8%)</td>
</tr>
<tr>
<td>Recurrence of Thalassemia n (%), day, post BMT</td>
<td>2 (18)</td>
<td>3 (20)</td>
<td>4 (9)</td>
<td>1 (7)</td>
<td>10 (9)**</td>
</tr>
<tr>
<td>Full donor chimerism, n(%)</td>
<td>5 (54)</td>
<td>10 (67)</td>
<td>31 (66)</td>
<td>10 (67)</td>
<td>57 (64)</td>
</tr>
<tr>
<td>Disease-free survival, n (%)</td>
<td>7 (64)</td>
<td>11 (73)</td>
<td>34 (72)</td>
<td>11 (73)</td>
<td>64 (72)</td>
</tr>
<tr>
<td>Overall survival n (%)</td>
<td>9 (82)</td>
<td>14 (93)</td>
<td>38 (81)</td>
<td>12 (80)</td>
<td>73 (83)</td>
</tr>
</tbody>
</table>

* Class I = 0, Class II=7, Class III=8. ** Class I = 1, Class II=3, Class III=5, BMT: Bone marrow transplantation

Conflict of Interest: None declared

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