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PRESENT AND FUTURE OF HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Books and other monographs [personal authors,[6,7] chapter in a book,[8] published proceeding paper,[9] abstract book,[10] monograph in a series,[11] agency publication[12]]:

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Seminars in Hematology

Present and Future of Hematopoietic Stem Cell Transplantation

May 17, 2002
Padua, Italy

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Seminars in Hematology

Present and future of hematopoietic stem cell transplantation

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Foreword

In the last ten years, recent discoveries on the biology of hematopoietic stem cells have laid the basis for promising future applications in almost all fields of medicine. Stem cells have became matter of discussion among scientists, politicians, experts on bioethics, newsmen and the general public because of the many implications of their use in the treatment of human diseases and their potential application in the field of preventive medicine and tissue-engineering. As a matter of fact, hematologists pioneered the clinical application of stem cells since many years ago with the introduction of hematopoietic stem cell transplantation for the treatment of many severe hematologic diseases. The impact of this application has been great and many patients have been able or, currently, hope to defeat their disease only through a hematopoietic stem cell transplantation. Ten years after its foundation, the Padua section of the Italian Association of Bone Marrow Donors (ADMO), and the Pediatric Hematology Oncology Clinic, which was the first pediatric Center to perform an allogeneic stem cell transplant in Italy, wish, with this congress, to assess the state of the art of this fascinating chapter of medicine and biology. The meeting gives ample space to illustrate the clinical results obtained so far and the next clinical applications but other aspects regarding the future new boundaries of stem cell technology will also be debated. We would like to thank the Rector of the University of Padua for allowing us to use the prestigious "Aula del Bo" for this meeting, all the invited speakers and all the people who have contributed to the realization of this important appointment.

Orietta Favaron
President of ADMO, Padua

Dr. Simone Cesaro
Scientific Secretary

Prof. Luigi Zanesco
Director of the Pediatric Hematology Oncology Clinic, Padua, Italy

Stem Cell Transplantation: Present and Future

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Advances in unrelated donor hematopoietic cell transplantation: improved matching and use of blood stem cells

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For patients without an HLA-identical sibling, transplantation of hematopoietic stem cells from HLA-compatible unrelated volunteer donors has become feasible thanks to the expansion of registries of HLA-typed volunteers that now include more than seven million individuals worldwide. The probability of matching patients with at least one donor for HLA-A, B and DR has increased as a function of the logarithm of the donor pool. In the National Marrow Donor Program of the United States, such a probability was 50% with a pool of 100,000 donors and has expanded to 85% with a pool greater than two million donors typed for HLA-A, B and DR. Utilization of unrelated donors as a source of hematopoietic stem cells has also increased because of improved safety of transplantation. The primary factor leading to improved patient outcome in the last decade has been the use of more precise and sensitive HLA typing using DNA-based techniques.

The role of HLA typing and matching on the outcome of unrelated donor transplantation

Results of a study of 1,874 unrelated donor marrow transplants facilitated by the National Marrow Donor Program of the United States was recently reported in abstract form.[1] Treatment regimens were selected by the transplant center. DNA samples from patient and donor were typed at the sequence level for HLA-A, B, C, DRB1, DQB1, DQA, DPB1 and DPA genes. The study revealed three major findings: 1) mismatch for HLA-A, B, C and DRB1 is associated with a worse survival, while mismatch for DQB1, DQA, DPB1 and DPA is not. Based on this finding, it is advisable that future donors will be screened for their matching, not only at HLA-A, B and DRB1, but also at HLA-C. Matching for DQ and DP genes remains of unproven effect on survival; 2) mismatching for one HLA-A, B or DRB1 DNA sequence disparity (allele mismatch) that is not recognized by anti-HLA antibodies is associated with decreased survival, and mismatching for one HLA-A, B and DR locus disparity that is recognized by antibodies (antigen mismatch) is associated with even worse survival. Thus, high resolution DNA typing at the *sequence level* is useful in selecting for more closely matched and safer donors. However, if a fully matched donor is not available, mismatch for an allele (i.e.: A*0101 vs. A*0102) is preferable to mismatch for an antigen (i.e.: A*01 vs. A*02); 3) mismatch for multiple alleles at HLA-A, B, C and DRB1 compounds the risk of mortality. This last finding confirms data in a prior report[2] and its implications are obvious.

The risk of graft failure is increased with donor disparity for HLA-A, B, or C and with patient homozygosity at the mismatched locus.[3] When donor and recipients differ for a single HLA locus, the risk of graft failure varies according to whether the incompatibility is for an HLA antigen or an HLA allele. In a study by Petersdorf *et al*. of patients transplanted from an unrelated donor, there were no episodes of rejection with a mismatch for a single allele (n=47), whereas rejection occurred in 14% of cases with a mismatch for a single antigen (n=51) and in 22% of cases when there was mismatch for multiple alleles (n=9). These data on graft failure from a single center are consistent with the data on survival from the National Marrow Donor Program, and demonstrate that mismatch for an antigen has worse clinical consequences than mismatch for an allele, and that the effect of mismatching for multiple alleles is cumulative.[1-3]

Modern HLA typing using DNA technology can distinguish subtle polymorphisms previously undistinguishable by classical serological typing

techniques. It is possible however, that demanding donor matching at the DNA sequence all for HLA-A, B, C and DRB1 loci will constitute an unnecessary stringency, and in some cases will prevent access to transplantation. The allowable limits of genetic disparity will likely differ according to the patient's underlying disease and stage. While patients with low risk disease and fair life expectancy in absence of transplant would want to avoid even the minimal risk associated with a mismatched donor, patients with high risk disease in advanced stage will likely have to tolerate the risk associated with the use of a donor mismatched for a single antigen or multiple alleles, rather than face the greater risks of the disease without transplantation. Therefore, the definition of an acceptable mismatch will require analyses of large number of patients with homogeneous disease risk.

Survival improvement trend over time

In Seattle, better donor matching and prophylaxis of cytomegalovirus disease and candida septicemia have resulted in improved survival in patients transplanted from an unrelated donor.[4] Patients transplanted for chronic myeloid leukemia in chronic phase between 1988 and 1991 (n=61) had a Kaplan Meier estimate of survival at five years of 49%, compared to 65% for patients transplanted between 1992 and 1998 (n=194, p=0.01). Best survival was observed in patients 18 to 40 years old (n=112) with a Kaplan Meier estimate of 79% at 5 years compared to 54% for patients 41-50 years old (n = 70, p = 0.002), and 20% for patients older than 50 (n=10, p=0.007). Patients above the age of 40 appear to tolerate high-dose whole body irradiation poorly. There is a report in abstract form of decreased morbidity and mortality, despite the use of an unrelated donor, in older patients receiving a regimen of fludarabine 90 mg/m^2 and low dose whole body irradiation 200 cGy.[5]

Role of stem cell dose

Patients with acute myeloid or lymphoid leukemia transplanted with unrelated donor bone marrow enjoyed a significantly improved survival when transplanted with a marrow cell dose greater than 3.7×10^8 nucleated cells per kg of body weight as opposed to a lower cell dose.[6,7] A subsequent single center study was conducted with the hypothesis that the reason for the improved outcome of recipients receiving a high marrow cell dose was related to the dose of CD34 cells.[8] The transplant center requested from the unrelated donor a marrow dose containing 4×10^8 nucleated cells per recipient body weight. In a cohort of 111 patients older than 20 years of age transplanted with T-replete marrow, the one-year survival was 66% if the CD34 cell dose was greater than 2.5×10^6 per kg of body weight, as opposed to 44% with a lower dose (p=0.003). The dose of CD4, CD8, or CD3 T-cells, B-cells, or monocytes did not affect the probability of a one-year survival in that study. By multivariable analysis, a higher CD34 cell dose was associated with an improved probability of sustained engraftment defined by neutrophils above 500/μL throughout the first 100 days, a lower risk of non-relapse mortality (hazard ratio 0.70, 95% confidence interval, 0.55-0.90, p=0.004) and less overall mortality (hazard ratio 0.79, 95% confidence interval, 0.66-0.94, p=0.008). These data suggest that human bone marrow is a limited source of hematopoietic progenitor cells for transplantation.

Since mobilization with granulocyte-colony stimulating factor (G-CSF) followed by blood cell apheresis can produce two to three fold higher number of CD34 cells, there is a rationale to testing the use of peripheral blood progenitor cells for transplantation. A single center study in Seattle has tested the use of peripheral blood stem cells (PBSC) from unrelated donors in patients with acute myeloid or lymphoid leukemia. A preliminary survival analysis in patients up to the age of 40 years transplanted in first or second remission (n = 29) is currently showing a Kaplan-Meier estimate of 73% at two years. The National Marrow Donor Program of the United States has launched a multicenter open-label phase II study to evaluate the use of G-CSF-mobilized peripheral blood stem cells in unrelated donor transplantation. A multivariate analysis has retrospectively compared the outcome of patients transplanted with PBSC or marrow over the same period of time at the same centers. The use of PBSC was associated with faster engraftment of neutrophils and platelets, with a suggestion for an increased incidence of acute graft-versus-host disease. The overall survival and disease-free survival were similar.[9] The relative benefits of unmodified bone marrow or peripheral blood components from unrelated donors will be tested in a randomized trial. Pilot studies are needed to test the use of modified components depleted of alloreactive T-cells with the goal of preventing GVHD.

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Bone marrow or peripheral blood as a source of stem cells for allogeneic transplantation

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Background and Objectives. Peripheral blood (PB) stem cell transplants are being increasingly used in the allogeneic setting, and are often preferred to the conventional bone marrow (BM) source. The aim of this report is to review available data on PB vs BM hematopoietic stem cell transplantation (HSCT). The discussion is restricted to unmanipulated HLA identical sibling transplants.

Evidence and Information Sources. Data with appropriate follow-up are available only for this type of comparison: we have preliminary data on the use of PB from unrelated donors, and on the use of T-cell depletion/CD34+ selection methods. The latter are rapidly evolving and it may be difficult to find a concurrent group of patients receiving T-cell depleted or CD34-selected marrow.

State of Art. The results of retrospective and prospective studies are quite similar: hematologic and immune recovery is faster after PB grafts, acute graft-versus-host disease (GvHD) is comparable, whereas chronic GvHD is increased in recipients of PB transplants. Transplant-related mortality (TRM) is similar in the two groups, whereas disease recurrence is lower after PB grafts.

Perspectives. The general feeling is that PB grafts are indicated for patients with advanced disease, whereas for early phase patients the two sources may give comparable results.

Key words: bone marrow, peripheral blood, allogeneic stem cell transplantation.

The first question pertains to the safety and tolerability of harvesting stem cells from the bone marrow (BM) under general anesthesia, or from the peripheral blood (PB) after treatment with 5-10 μg/kg/day granulocyte colony-stimulating factor (G-CSF) for 4-5 days. PB harvest does not involve a general anesthesia, and this is certainly an advantage. However the leukapheresis can be cumbersome at times, and in very young donors (<5 years) this may be a problem. Complications such as thrombocytopenia,[1] splenic rupture[2] and cerebral vascular episodes have been reported after PB harvest. On the other hand the major drawback to marrow harvest is general anesthesia, with a risk of fatal complications of approximately 1:10000. A recent study from the *National Marrow Donor Program* identified 8 deaths in healthy stem cell donors: 6 were marrow donors and 2 were G-CSF-primed peripheral blood donors, giving an overall risk of 1:15/20.000 (or 0.007%).[3] Therefore the two harvest procedures seem to be equivalent as far as concerns the risk of fatal complications. Early non-fatal complications are different but again equivalent in terms of numbers and severity. Peripheral blood harvest is still not universally accepted by *National Donor Registries*, mainly because of concern on long-term effects of G-CSF treatment in healthy volunteers. A recent study by Cavallaro *et al.*,[4] based on a questionnaire sent to all PB donors, suggests that PB donors were well and free of major complications 3-6 years after peripheral blood stem cell donation.

The information we have at present suggests that stem cell harvest from the marrow or the peripheral blood is a procedure which is well tolerated by donors. Both types of harvest are associated with an extremely low risk of fatal complications, which should be covered be an appropriate insurance. The level of insurance coverage varies greatly from one country to another. The

World Marrow Donor Association (WMDA) is working on a *bill of rights* for stem cell donors, which should include harvest procedures, insurance coverage, pre- and post-donation counseling and monitoring. It is hoped that we will soon have common procedures for stem cell donations worldwide, since donors have the right to be protected to the same degree in every donor center.

Composition of the graft

Compared to bone marrow grafts, PB transplants contain significantly more cells and more $CD34^+$ cells. However, the most striking difference is the number of $CD3^+$ cells which is almost one log higher in PB stem cell transplants. The other striking difference is the number of monocytes, as reported in the randomized French study, since PB grafts contain 30 times more monocytes than do BM grafts. The last difference is the lack of mesenchymal stem cells (CFU-F) in PB collections,[5] whereas in a conventional BM transplant the number of infused stromal cells is 1×10^4/kg.[6] Therefore PB and BM transplants have different compositions and PB collections contain significantly more $CD34^+$ cells (×2), more $CD3^+$ cells (×10), more monocytes (×30) and no stromal cells or CFU-F.

Speed of hematopoietic recovery

Hematologic recovery is faster after PB stem cell transplants than after BM ones and this includes neutrophil, platelet and, possibly, red blood cell recovery. This has been shown by retrospective and prospective studies, with no exception. The median day to 0.5×10^9 neutrophils depends on whether GvHD prophylaxis includes methotrexate (MTX) or not. However there is a reduction of 3 days in the time to 500 neutrophils, this being day +17 for BM and day +14 for PB stem cell transplants. Time to 30×10^9/L or 50×10^9/L platelets is also significantly reduced in PB graft recipients, and platelet counts on day +21 are significantly higher in these patients.

Therefore there is conclusive evidence that PB transplants accelerate hematologic reconstitution, and this of course is something highly desirable after a hematopoietic stem cell transplant.

Transfusion requirements

Although the number of red blood cell transfusions is similar in most studies, there is a significant reduction in the need for platelet transfusions in recipients of PB grafts: in one randomized study the figures were 6 transfusions for PB recipients and 11 for BM recipients ($p<0.006$).[7]

Quality of hematopoietic recovery

Unfortunately few studies take into consideration the quality of hematologic recovery in the medium/long-term. It is well-known that after the initial engraftment the peripheral blood counts of many patients drop between day +50 and +150, possibly in association with GvHD, cytomegalovirus infections and/or ganciclovir treatment. In our comparative study[8] we found no difference in platelet counts between BM and PB recipients beyond day +21. We have recently updated this study on 310 HLA identical sibling transplants: there was a significantly better platelet recovery by day 21 in PB recipients ($p=0.0001$) (33 vs 22×10^9/L), but the advantage was lost by day 50 and thereafter. It is also interesting that 2 years post-transplant there was a borderline higher platelet count in patients receiving BM (186 vs 162×10^9/L, $p=0.08$) and also between 2 and 5 years (210 vs 188×10^9/L, $p=0.07$) possibly associated with more chronic GvHD in the PB recipients.

These data might suggest that, despite the administration of a significantly larger number of cells, the quality of marrow function is similar in BM and PB stem cell recipients: whether this is due to the lack of stromal cells in PB grafts or to increased GvHD remains to be determined.

Immune recovery

The number of studies looking at immune recovery is small. In our study the recovery of $CD4^+$ cells was faster for PB graft recipients than for BM transplant patients. The former group achieved a $CD4^+$ count of > 200 cells early after transplantation, whereas BM transplant recipients reached this level more than one year after transplantation. The recovery of $CD3^+$ and $CD8^+$ was also faster, whereas recovery of $CD16^+$ cells was similar. In a study by Ottinger *et al.*[9] the numbers of naive ($CD4^+CD45RA^+$) and memory cells ($CD4^+CD45R0^-$) helper cells were higher after PB transplants ($p=0.003$ and 0.001, respectively). The faster immune recovery does not translate into a reduced incidence of infections: this is particularly strange for cytomegalovirus (CMV), since one would expect this complication to be controlled by the large number of circulating $CD4^+$ cells.

Cell dose effects

Several studies looking at BM transplant recipients have reported a cell dose effect: *more is better*.[10-12] It is interesting that in his first report (1977) Storb suggested that *the greatest possible dose of marrow should be obtained perhaps supplemented by stem cells derived from the peripheral blood*. In

PB transplants, on the contrary, *more seems to be worse*.[13] This could be because many cells in unmanipulated PB grafts also means many more T-cells and more acute GvHD. If we want to increase the cell dose in hematopoietic stem cell transplants we may need to use the currently available manipulations such as $CD34^+$ selection, in addition to a conventional BM transplant.

Acute graft-versus-host disease

The first comparative study was published by the Seattle group in 1996.[14] That study included 74 patients who received BM (n=37) or PB (n=37). The authors reported a comparable rate of acute GvHD. We confirmed this finding in our series of 97 patients:[8] 54% vs 42% of patients developed grade 0-I acute GvHD (p=0.15), 38% vs 48% grade II (p=0.2), and 8% vs 9% grade III-IV (p=0.1) for marrow vs blood grafts, respectively. The actuarial probability of developing acute GvHD grade ≥ II was 47% vs 58% in BM vs PB recipients (p=0.3). The median interval from transplant to GvHD was 16 vs 17 days, respectively (p=0.6).

Many other retrospective studies as well as the prospective randomized studies have also confirmed that acute GvHD is comparable after PB and BM transplants. One recent study points to a slightly earlier onset of acute GvHD[7] and a slightly increased severity. It is noteworthy that the one log difference in T-cell content between BM and PB grafts does not translate into different acute GvHD. One explanation could be that T-cells exposed to G-CSF are polarized towards T^2 anti-inflammatory functions.[15]

Chronic graft-versus-host disease

The fist report on the increased risk of chronic GvHD after allogeneic PB grafts came from Majolino *et al*.[16] This early observation based on 6/9 patients developing chronic GvHD has since been confirmed in the large retrospective EBMT/IBMTR study.[17] The difference is not seen early after transplants but becomes more evident at one year and later.[17] Other retrospective and prospective studies, with a few exceptions, also report a higher incidence of chronic GvHD, possibly accompanied by an increased severity. The recent co-operative Spanish study, looking specifically at patients alive on day +100 and eligible to develop chronic GvHD, suggests that most if not all patients receiving unmanipulated PB transplants are at risk of developing chronic GvHD. Chronic GvHD has opposing effects on outcome: on one hand it protects against relapse, on the other it increases the risk of late complications and transplant-related deaths. The protective effect on relapse first described in the early eighties[18] can translate into a survival advantage: this is true especially in patients with acute lymphocytic leukemia (ALL), in whom post-transplant relapse leads to a very high risk of death.[19] The beneficial graft-versus-leukemia (GvL) effect of chronic GvHD is less clear in chronic myeloid leukemia (CML) patients, for whom effective treatment of relapse with donor lymphocyte infusion (DLI) now exists. In its extensive form chronic GvHD leads to late complications such as infections and secondary tumors.[20] Therefore it may be desirable to induce only limited and not extensive chronic GvHD. In a recent analysis we looked at secondary tumors in 804 patients undergoing allogeneic stem cell transplants and surviving at least 100 days. The median follow-up was 1,054 days (101-8,590) and 2,131 for surviving patients. The diagnosis was aplastic anemia (n=68), acute leukemia (n=345), chronic myeloid leukemia (n=277), lymphoma (n=35), chronic myeloproliferative disorders (n=13), and myelodysplasia (n=66). The total number of secondary tumors was 25. The actuarial risk of developing a secondary malignancy at 10 years was 7±12% and at 15 years 13±20%. The risk was similar for patients given total body irradiation (TBI) and for patients given alkylating agents such as busulfan or thiotepa. The strongest predicting factor was chronic GvHD: patients with no, limited or extensive chronic GvHD had a risk of secondary tumor at 10 years of 3±14%, 4±14% and 14±20%, respectively (p<0.0001) (*unpublished data*). Because more allogeneic PB recipients than allogeneic BM graft recipients seem to have extensive chronic GvHD,[17] one would expect the incidence of secondary tumors to rise in the next 10 years in PB graft recipients.

Finally some authors have quoted a reduced quality of life, with lower Karnowski's scores in allogeneic PB recipients.[21] This may also be due to the fact that patients with persisting chronic GvHD need to be treated long-term, and perhaps lifelong with immunosuppressive therapy, usually a triple association, including prednisone, cyclosporine and azathioprine. This in turn seems to further increase the risk of secondary tumors.[22]

Therefore, the evident protective effect of PB transplants on relapse is achieved at the expense of more chronic GvHD. Because some of the complications may require 10 to 15 years to develop, we will need to follow these patients for a long time before we can draw definitive conclusions on the relative benefit of using either BM or PB grafts.

Infections and hospital stay

Time spent in hospital and days with intravenous antibiotics are reported to be reduced in PB recipients in some but not all studies: the average number of days of intravenous antibiotics was 16 for BM and 13 for PB recipients (p value significant in 1/3 studies), and the time spent in hospital 32 vs 26 days (p value significant in 2/5 studies). There was no apparent reduction in the incidence of CMV antigenemia (41% for BM and 51% for PB graft recipients, p=ns). Unfortunately the largest retrospective study (the IBMTR/EBMT analysis) does not report these data. One can conclude that there seems to be a trend towards a shorter stay in hospital with PB transplants, but the infectious complications are comparable; the duration of antibiotic treatment was shorter in PB recipients in 1/3 studies.

Transplant-related mortality, relapse, and survival

The average transplant-related mortality was 27% for BM recipients vs 24% for PB in 6 retrospective studies and 23% vs 21% in 3 prospective trials. All studies reported comparable TRM for BM and PB recipients. Relapse was lower in 2 prospective randomized studies (one of which is unpublished): relapse was also lower in the retrospective IBMTR/EBMT study for patients with advanced CML. Survival, given different diagnoses and different phases of disease, was comparable in all studies. The IBMTR/EBMT retrospective study showed a survival advantage for patients with advanced CML receiving PB cells.

In our single center retrospective study the overall actuarial 3-year TRM for BM vs PB patients was 20% vs 33% (p=0.1), survival was 53% vs 48% (p=0.3), and relapse was 42% vs 43% (p=0.8). For patients in first complete remission these figures were: TRM 12% vs 22% (p=0.2), survival 75% vs 70% (p=0.4) and relapse 31% vs 9% (p=0.4).

Conclusions

Donation of hematopoietic stem cells via marrow harvesting or G-CSF priming and leukapheresis is a relatively safe procedure: this is true for young and healthy individuals. However complete information on adverse events and insurance coverage should be provided. The composition of unmanipulated BM and PB grafts is quite different: PB grafts contain almost one log more T-cells, more monocytes, more $CD34^+$ cells and no stromal cells. In addition the cells have been exposed to G-CSF and this modifies lymphoid and hematopoietic cells, possibly to the better. Acute graft-versus-host disease is comparable, whereas chronic GvHD is usually increased after PB transplants: this has a beneficial effect on relapse, though a possibly detrimental effect on quality of life and late complications. We should not forget that G-CSF-mobilized PB cells are, at present, being increasingly used after manipulation and for mismatched transplants: we need to explore these new avenues as they may change the way we prepare the graft for hematopoietic stem cell transplantation between HLA identical siblings.

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Full haplotype mismatched hematopoietic stem cell transplants

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Background and Objectives. Clinical outcome in leukemia patients transplanted from one-haplotype mismatched donors has been largely disappointing because of the high incidence of severe graft-vs-host disease in T-replete transplants or high rejection rates in T-cell-depleted transplants.

Evidence and Information Sources. The breakthrough came with the use of a megadose of T-cell-depleted progenitor cells after a high intensity conditioning regimen.

State of Art. Today, high risk acute leukemia patients are treated at less advanced stages of disease, receive a well-tolerated conditioning regimen, and benefit from advances in post-transplant immunologic reconstitution. Overall, event-free survival and transplant-related mortality compare favorably with those reported for unrelated matched transplants.

Perspectives. T-cell depleted megadose stem cell transplant from a mismatched family member, who is immediately available, can be offered as a viable option to candidates with high-risk acute leukemias.

Key words: haploidentical transplant, acute leukemia.

Although bone marrow transplantation offers a cure to many patients with leukemia or other malignant hematologic diseases a matched sibling donor is not always available and in the World Registries of unrelated donors the chances of finding a match range from 60-70% for Caucasians to under 10% for ethnic minorities.[1,2] Time is also a crucial factor for patients who urgently need transplants because they may be lost while the Registry search proceeds and the bone marrow cells are harvested. On the other hand practically all patients have an HLA-haploidentical two- or three-loci mismatched family member who is immediately available as a donor. Clinical experience with haploidentical mismatched transplants started some twenty years ago but unfortunately transplanting unmanipulated bone marrow from these donors was largely unsuccessful because of the high incidence of severe graft-versus-host disease (GvHD).[3]

Several clinical trials in HLA-identical transplants demonstrated that extensive T-cell depletion of the bone marrow prevented acute and chronic GvHD without the need for any post-transplant prophylaxis and did not impair engraftment.[4,5] These findings were confirmed in SCID patients who received a transplant from HLA haploidentical three loci mismatched relatives.[6] Unfortunately when tested in leukemia patients this type of transplant resulted in a high incidence of graft failure.[7]

Megadose transplantation

The turning point in the history of T-cell-depleted mismatched transplants was the clinical application of a megadose of stem cells.[8] This principle was successfully tested in a series of mouse models in the late 1980s which showed that escalating doses of T-cell-depleted mismatched bone marrow cells were associated with full donor type engraftment.[9] In 1993 we applied the cell-dose escalation concept for the first time clinically in 36

Table 1. Clinical characteristics of patients.

	AML	*ALL*	*Total*
No. of patients	32	17	49
M/F	15/17	8/9	23/26
Age median (range)	38 (17-62)	23 (9-46)	26 (9-62)
Status at transplant			
CR I	4	6	10
CR ≥ II	13	3	16
Relapse	15	8	23

Table 2. Conditioning regimens.

Patient day	*Regimen*
9	TBI 7.5 Gy in a single fraction (4 Gy on the lungs)
8	Thiotepa 5 mg/kg (4h-infusion)
7	Thiotepa 5 mg/kg (4h-infusion)
6	Fludara 40 mg/m^2 in 4h infusion + rATG 5 mg/kg (8h-infusion)
5	Fludara 40 mg/m^2 in 4h infusion + rATG 5 mg/kg (8h-infusion)
4	Fludara 40 mg/m^2 in 4h infusion + rATG 5 mg/kg (8h-infusion)
3	Fludara 40 mg/m^2 in 4h infusion + rATG 5 mg/kg (8h-infusion)
2	Rest
1	Rest
0	HSCT

adults with advanced leukemia.[10] Bone marrow was supplemented with peripheral blood progenitor cells after mobilization with granulocyte-colony stimulating factor (G-CSF). Both sources of stem cells were depleted of T-cells by soybean agglutination and E-rosetting. Recipients were conditioned with highly immunosuppressive and myeloblative regimens (total body irradiation TBI) in a single fraction at a fast dose rate, cyclophosphamide, antithymocyte globulin and thiotepa). Eighty percent of patients achieved primary sustained engraftment and only 18% developed grade II to IV acute GvHD, even though no post-transplant immunosuppressive therapy was given.

Modifications to our approach led to remarkable progress. In October 1995, in an attempt to minimize the extra-hematologic toxicity of the TBI-based conditioning protocol, fludarabine was substituted for cyclophosphamide.[11] At the same time, we started to select CD34$^+$ cells positively, using either the Ceprate system (43 patients) or, after January 1999, the CliniMacs device (49 patients).[12,13] Furthermore, in this latter group of transplanted patients, we did not administer G-CSF after transplant because experimental data and preliminary reports suggested that it induces immunosuppression.[14,15]

Work in progress

We report here the transplantation outcome in these last 49 high-risk acute leukemia patients (32 with acute myeloid leukemia-AML and 17 acute lymphocytic leukemia-ALL), all of whom benefitted from our recent technical improvements (Table 1). There were 23 males and 26 females. Ages ranged from 9 to 62 years with a median of 38 years for the patients with AML and 23 for those with ALL. It is worth noting that 14 were in the upper age group (between 45 and 62 years) for transplant. All these patients were at high risk of leukemia relapse: 23 (47%) were actually in relapse at the time of the transplant, 16 (33%) in complete remission (CR) ≥ II and even the 10 (20%) in CR I were at high-risk because of unfavorable prognostic features at diagnosis: complex karyotypes (n=2), minus 7 (n=1), t 8;12 (n=1), t 9;22 (n=2), t 4;11 (n=1), minus 13 (n=1), leukemia-lymphoma (n=1) and slow remission (n=1). Because of the history of the disease and the stage at transplant 39/49 were at high-risk of transplant-related mortality.

After our TBI-based conditioning protocol (Table 2), patients received a median of 12 ×10^6 CD34$^+$ cells/kg and 1.5×10^4 CD3$^+$ cells/kg. Primary full-donor engraftment was achieved in 45/49 (92%) patients. All except one of the other four successfully engrafted after secondary transplants from different haploidentical family donors. Hematopoietic recovery was extremely rapid with neutrophil counts reaching 1 ×10^9/L and platelet counts 25 × 10^9/L at a median of 13 days (range 8-19) and 19 days (range 12-84), respectively. Even though no post-transplant immunosuppressive therapy was given, acute GvHD grade ≥ II occurred in only 3 cases (Table 3).

Without receiving any post-transplant G-CSF, immunologic reconstitution markedly improved and CD4$^+$ cell numbers rose to 100 and 300/mm^3 at 60 and 180 days post-transplant, respectively. Most of the post-transplant CD4$^+$ T-cell clones exhibited protective Th1/Th0 cytokine production features, i.e., all clones expressed functional interleukin (IL)-12 receptors and few produced IL-4 and IL-10. These patterns were very different to those that had been observed in recipients who had received G-CSF, whose CD4$^+$ clones had clearly exhibited non-protective type-2 functional features.[15]

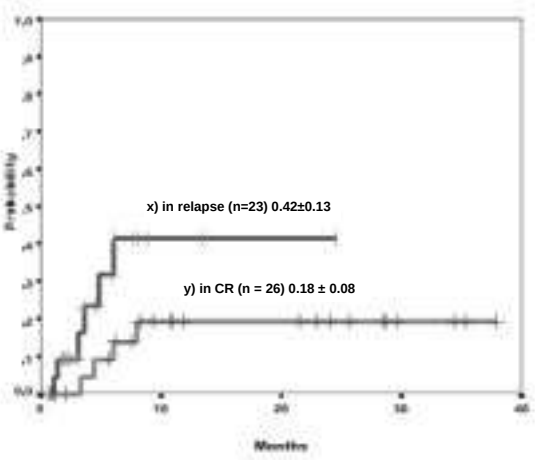

Figure 1. Infection-related mortality.Patients = 49; Disease = AML: 32, ALL:17; Status = CR1:10; CR≥2:16; Relapse = 23.

Table 3. Results.

Infused cells (median)	
CD $34^+ \times 10^6$/kg	12
CD $3^+ \times 10^4$/kg	1.5
Engraftment	48
Primary	45
Secondary	3
Median days to:	
ANC > 1×10^9/L	13
PLT > 25×10^9/L	19
Acute GvHD≥ II	3

The improved immunologic reconstitution may have contributed to the low probability of infection-related deaths (0.18±0.08) in the 26 patients who were in any CR at transplant (Figure 1). Overall, 16 patients have died of non-leukemic causes, 12 from infections and 4 from other causes (leukoencephalopathy, GvHD, rejection and idiopathic interstitial pneumonia). The probability of transplant-related mortality was 0.32 and 0.47 in the 26 patients in CR and in the 23 in relapse, respectively.

The probability of leukemia relapse was 0.25 for the 26 patients in any CR at transplant and 0.87 for the 23 in relapse. We had recently observed in *in vitro* leukemia killing assays that donor-vs-host natural killer (NK) cell alloreactive clones lyse AML blast cells.[16,17] Therefore we re-analyzed the AML transplants according to whether they had the potential for donor NK alloreactivity. Strikingly, only one patient of the 15 with donor NK cell alloreactivity has relapsed to date.

At present 20 patients (15/32 AML, 5/17 ALL) survive disease-free at a median follow-up of 23 months (range 1-38). The probability of event-free survival is 0.74 and 0.37 for AML and ALL patients, respectively, in any CR at transplant (Figure 2).

The results that we have achieved with our current transplant strategy indicate that mismatched transplant should be offered to high-risk acute leukemia patients without an HLA-identical donor not as a last resort, but as a viable option in the early stages of the disease.

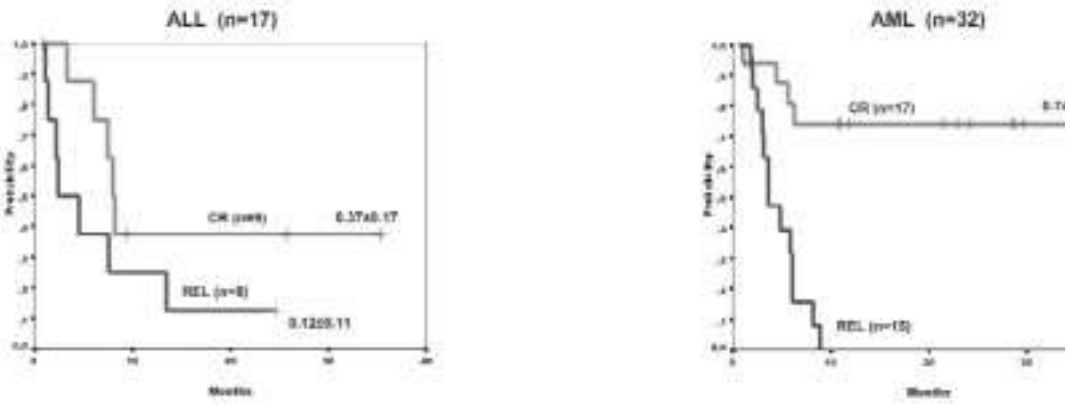

Figure 2. Event-free survival.

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Non-myeloablative allogeneic hematopoietic stem cell transplants

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Conventional allogeneic hematopoietic stem cell transplantation (HSCT) has been increasingly used over the last 30 years to cure many hematologic diseases.[1] This strategy was founded on the principles of maximal tumor cytoreduction, according to the well-demonstrated dose-response relationship of chemoradiotherapy, and of adequate immunosuppression, to permit engrafment even after non-HLA-genotypically identical donor transplants.[2-4] Despite maximally tolerated doses of chemoradiotherapy, relapse probabilities remain high.[4-7] High-dose chemoradiotherapy is also associated with substantial transplant-related toxicity and a significant incidence of acute and chronic graft-versus-host disease (GvHD).[8] Because of these characteristics, allogeneic HSCT is offered only to relatively young patients (< 55 years old) with optimal organs and general performance status.[1]

There is a large amount of evidence that the donor stem cells may exert not only a repopulating role but also a graft-versus-tumor effect (GvT) due to recognition of malignant host cells.[9-11] Based on these data, attempts have recently been made to diminish transplant-related morbidity and possibly mortality by administering relatively non-toxic, non-myeloablative doses of chemotherapy or radiation therapy prior to allogeneic transplantation,[12-15] thus permitting the treatment of older patients and patients with medical infirmities. The main aim of this new strategy is to create a state in the patient in which the host's and the donor's hematopoietic systems co-exist (mixed chimerism).[12] The induction of mixed chimerism, moreover, may serve as a platform for the development of a graft-versus-tumor effect. To date, many approaches have been proposed in order to achieve this goal demonstrating that while it is possible to achieve a stable mixed chimerism after a non-myeloablative allogeneic transplantation, the effectiveness of these approaches, in terms of disease control, remains to be determined.

This review will briefly describe the preclinical evidence for these non-myeloablative transplant strategies, describe preliminary clinical experience and discuss the rationale for considering such approaches as transplant strategies.

Preclinical data

The observation that in some severe non-malignant disorders such as β-thalassemia, sickle cell disease, aplastic anemia, and autoimmune diseases, persistence or establishment of a state of mixed hematopoietic chimerism conferred an important clinical benefit[16-17] led many centers to investigate whether establishing a state of mixed chimerism would be possible in hematologic malignancies. This would have been the starting point of a strategy to treat malignancy by exploiting an allogeneic graft-versus-tumor effect.

Systematic *in vitro* and *in vivo* studies have been carried out in Seattle and Boston to find possible strategies to achieve a state of stable mixed chimerism.[18-21]

In a murine model, stable mixed lymphohematopoietic chimerism was achieved following low-dose total-body irradiation (TBI) (300 cGy) or cyclophosphamide (200 mg/kg), peri-transplant monoclonal anti-T-cell antibody, thymic irradiation and fully mismatched donor bone marrow transplantation (BMT).[20,21] With the addition of post-transplant cyclosporine (CYA), these mice were completely protected from acute and chronic GvHD. Remarkably, these animals were also resistant to the induction of GvHD following delayed donor lymphocyte

infusion (DLI) (beginning on day +35 post-transplant), despite a potent lymphohematopoietic graft-versus-host response which converted their state of mixed chimerism to one of fully donor hematopoiesis.

Studies performed at the Fred Hutchinson Cancer Research Center in dogs formed the preclinical milestone for a clinical translation of a mixed chimerism approach, based on the premise that a powerful post-graft immunosuppressive regimen would not only prevent GvHD but also host-versus-graft reactions.

In an attempt to obtain mixed chimerism in MHC-matched littermates non-lethal doses of 200 cGy TBI and the use of post-transplant CYA alone were not sufficient to establish stable mixed chimerism. The combination of methotrexate (MTX) and CYA was somewhat more effective (with at least 2 out of 6 animals becoming stable mixed chimeras). The most effective combination appeared to be CYA and mycophenolate mofetil (MMF) (11 out of 12 dogs).[19,20]

Preliminary clinical data

With this positive preclinical experience as a background, the clinical investigation of non-myeloablative transplant regimens began in a number of transplant centers. Several published reports have demonstrated the feasibility of achieving allogeneic engraftment following non-myeloablative conditioning therapy.[12-15] These reports indicated the tolerability of most of these regimens, and showed that mixed lymphohematopoietic chimerism can be intentionally induced, even across major HLA barriers. In some cases mixed chimerism led to a potent antitumor response, and that represented a particular important proof of principle in the field of clinical allogeneic stem cell transplantation.

Based on their dog model showing that mixed chimerism is reliably achieved following low-dose total body irradiation (200 cGy) and post-transplant immunosuppression (MMF and CYA), more than 156 patients with hematologic malignancies, ineligible for conventional allografting due to age, prior therapy or organ dysfunction were treated in Seattle. Seventy-three patients were conditioned with 200 cGy TBI alone, and 18% experienced non-fatal graft rejections. With the addition of fludarabine, rejections have become the exception. Most patients did not need platelet transfusions, and a few received red blood cell transfusions. The majority of HSCT were carried out entirely in the outpatient setting. Typical side effects of HSCT, such as alopecia, mucositis, diarrhea, and veno-occlusive disease of the liver, were absent.[22]

There were significantly fewer bacterial infections than seen after conventional HSCT.[23] Grade II-IV acute GvHD occurred in 57% of patients, with 37% having grade II, 13% grade III, and 7% grade IV disease. Chronic GvHD was seen in 65% of patients; however, it responded well to therapy. Fatal progression of underlying diseases occurred in 18% of patients. Non-relapse mortality at 1 year was 20%. With a median follow-up of 220 (range 100-1026) days, 62% of patients were alive, and progression-free survival was 50%. Complete remissions generally occurred slowly over periods of months.

Based on this first experience, the Seattle group started single disease protocols and also the unrelated non-myeloablative program. Among the different diseases, the results achieved in multiple myeloma deserved great consideration by the scientific community. In a multicenter phase II trial 32 patients with previously treated stage II/III myeloma were treated with autologous HSCT followed by a non-myeloablative allogeneic HSCT from HLA-identical siblings according to the Seattle regimen. Thirty-one of the 32 patients received non-myeloablative allogeneic HSCT with medians of 0 days of hospitalization, neutropenia and thrombocytopenia. TRM at day 100 was 6% (one death after autologous HSCT and one from progressive disease after allogeneic HSCT). Forty-five percent of patients developed acute grade II-IV GvHD, and 55% developed chronic GvHD requiring therapy. The response rate was 84% with 53% CR and 31% PR and only two progressions to date. This study provided the rationale for a phase III trial comparing standard autologous HSCT to this two-step allogeneic approach.[24]

The first reports on the unrelated program, despite a not negligible rejection-rate (11%), confirmed the feasibility and safety of the Seattle regimen in this setting of transplants as well. Remarkably, many patients with chemorefractory hematologic disease achieved tumor control after this approach.[25]

Using a similar non-myeloablative preparative regimen to that used in the murine model of Sykes *et al.*,[20,21] the Boston group induced mixed lymphohematopoietic chimerism in patients with chemoradiotherapy-refractory hematologic malignancies.[26] Twenty-eight patients received an HLA-matched donor transplant while 16 received an HLA-mismatched donor transplant. Of 23 evaluable recipients of HLA-matched donor transplantation, 20 have achieved stable mixed lymphohe-

matopoietic chimerism. Ten patients with stable mixed chimerism, who had no evidence of GvHD, received DLI beginning on day +35 post-transplant. Conversion of mixed chimerism to full donor hematopoiesis occurred in six of the ten patients. Full donor T-cell chimerism was not necessary for the development of acute GvHD (or antitumor response). Notable antitumor responses have been seen in the majority of these patients with refractory hematologic malignancies (7/23 evaluable patients with chemorefractory Hodgkin's disease or NHL achieved a partial remission and eight a complete response). Twenty-two patients are reported to be alive. Thirteen of these 22 patients were evaluable for response, and eight were clinically progression free. The incidence of acute GvHD grade ≥ 2 was low (29%) allowing for the early administration of DLI. TRM was 10%. Several patients have had a conversion of chimerism and achieved complete remission without the development of severe GvHD.

Recently the M.D. Anderson Cancer Center group published the results of 3 subsequent trials of reduced intensity conditioning with melphalan (180 mg/m^2) and purine analogs (mostly fludarabine 125 mg/m^2) for the treatment of hematologic malignancies.[27] Eighty-six patients (of whom 8 received cladribine instead of fludarabine) considered ineligible for conventional allogeneic SCT were treated according to that regimen. Forty of these patients received their graft from a matched unrelated donor. The status at transplant was 1st remission (n = 7), untreated 1st relapse or subsequent remissions (n = 16) and refractory disease (n = 63). Eighty patients had donor cell engraftment between 80% and 100% by day 30. Acute GvHD prophylaxis was tacrolimus and methotrexate (5 mg/m^2); the probability of grade II-IV acute GvHD was 0.49 and 16/41 deaths before day 100 were due to GvHD. The risks of developing acute GvHD and dying were higher in the unrelated group (62% vs 41% and 11/40 vs 4/46, respectively). The overall 2-year survival probability was 0.28 for all patients. For patients in 1st CR and for those in untreated 1st relapse or subsequent remissions disease-free survival was 57% and 49%, respectively. TRM at day 100 was 37.4% for the fludarabine-treated group while it was 87.5% for the cladribine group.

Khouri *et al.*[28] treated 20 patients with follicular or small cell lymphocytic lymphoma after relapse from a prior response to conventional chemotherapy. The preparative regimen was fludarabine (25 mg/m^2 given daily for 5 days or 30 mg/m^2 daily for 3 days) and intravenous cyclophosphamide (1 g/m^2 given daily for 2 days or 750 mg/m^2 daily for 3 days). Thirteen patients received peripheral blood stem cell transplants from HLA-identical sibling donors. Nine patients received rituximab in addition to chemotherapy. Hematologic recovery was prompt and sustained in all patients and none developed graft failure. All patients had evidence of donor cell engraftment; the median percentage of donor cells at 1 month after transplantation was 80% (range, 10-100%). The cumulative incidence of acute grade II to IV GvHD was 20%. Chronic GvHD developed in 8 patients. All patients achieved a complete remission. None of the total group has relapsed. The median follow-up period was 21 months (5-46 months). Seventeen patients (85%) remain alive and in complete remission.

Using a busulfan-based preparative regimen, Slavin *et al.*[14] demonstrated excellent tolerability and favorable survival probabilities in 26 patients with hematologic malignancies and four patients with genetic diseases. Preparative therapy consisted of busulfan at a dose of 8 mg/kg, plus fludarabine 180 mg/m^2 and anti-T-lymphocyte globulin. GvHD prophylaxis consisted of CYA. Twenty-five patients received HLA-identical sibling donor transplants. One patient received stem cells from a donor with a single antigen mismatch at the A and C locus. Treatment was generally well tolerated. All patients had evidence of donor engraftment. In 9 of 26 evaluable patients, transient mixed chimerism was observed. Acute GvHD occurred in 12 of 26 patients. Six patients developed grade III-IV GvHD. Limited chronic GvHD developed in 9 of 25 evaluable patients. At a median of eight months post-transplant, 22 of 26 patients (85%) were alive, 21 of whom (81%) were clinically disease free.

Bacigalupo[29] explored a regimen with thiotepa (10 mg/kg × 1 day) and cyclophosphamide (50 mg/kg × 2 days) in 33 patients with a median age of 52 years (range 4-60) transplanted for hematologic disease from identical siblings. The source of hematopoietic stem cells was bone marrow (n=17) or granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood (PB) (n=16). GvHD prophylaxis consisted of CYA and a short course of methotrexate. Acute grade III-IV GvHD occurred in 3% of patients. Chronic GvHD was seen in 45% of patients, with a significant difference between PB (69%) and BM transplants (23%) (p=0.009). For BM grafts the actuarial 2-year TRM was 6%, relapse was 56% and survival 87%; for PB grafts, these figures were, 27%, 33%, and 68%, respectively. Twenty-five patients are alive at a median follow-up of 762 days (range

216-1615) and 20 patients (60%) remain disease-free. Thirteen patients (39%) received donor lymphocyte infusion (DLI) either for persisting or relapsing disease and 6 patients had complete remission. Corradini *et al.*[30] described the engraftment kinetics after non-myeloablative therapy with thiothepa (10 mg/kg ×1 day), cyclophosphamide (30 mg/kg/d ×2 days) and fludarabine (25 mg/m^2/d ×2 days) and HLA-matched or one-antigen mismatched donor blood stem cell transplantation in 45 patients with hematologic malignancies. Patients who did not achieve clinical and molecular remission were eligible for monthly escalating doses of DLI. GvHD prophylaxis consisted of CYA and methotrexate. All patients engrafted. The probability of grades II-IV and III-IV acute GvHD was 47% and 13%, respectively. The probability of non-relapse mortality, progression-free survival, and overall survival was 13%, 57%, and 53%, respectively.

Recently another Italian group published[31] the results of a combined transplant approach autografting followed by a non-myeloablative allograft in 15 patients with advanced resistant Hodgkin's and non-Hodgkin's lymphoma. At a median of 61 days after autotransplant patients were conditioned with cyclophosphamide (300 mg/m^2/day) and fludarabine (30 mg/m^2/day) for 3 days and received peripheral stem cells from HLA identical sibling. CYA and MTX were given as GvHD prophylaxis. All patients promptly engrafted. Seven patients developed acute GvHD and 2 extensive chronic GvHD; TRM was 13%. Five patients were in continuous CR at the time of the report. The combined approach was reported to be safe and have a high response rate.

Solid tumors

Because of the significant TRM following allogeneic HSCT, few investigators have performed allogeneic transplants in non-hematologic malignancies. Two groups investigated the use of allogeneic HSCT in breast cancer.[32,33] In both cases evidence of a graft-versus-breast cancer effect was reported. These preliminary reports suggested the existence of a GvT effect also in solid tumors but the toxicity of the approach stopped further investigations in this field. Non-myeloablative allogeneic transplants with their low-toxicity profile offer possibilities for this hypothesis to be explored in greater detail.

Childs *et al.*[34] achieved regression of tumor in 10/19 (53%) patients with metastatic renal cell carcinoma who were treated with an HLA-identical sibling allogeneic HSCT. TRM was 10%. The median interval of four months from pretransplantation preparative chemotherapy to the first signs of disease regression, the observation that regression occurred only after complete donor T-cell chimerism had been established, and the association of graft-versus-host disease with regression of metastases were all consistent with the occurrence of an antitumor effect that was mediated by the donor's T-cells. These results led many centers to start non-myeloablative allogeneic HSCT protocols in solid tumors. Some preliminary data have been published in abstract form and most of them confirm the existence of a GvT effect also in solid tumors.

Other applications of non-myeloablative preparative regimens for allogeneic stem cell transplantation

Given the excellent tolerance of these non-myeloablative regimens and the high rates of alloengraftment, even following transplants from HLA-mismatched donors, there has been considerable interest in extending these transplant strategies to patients with non-malignant disease.[14,35,36] These include the genetic diseases described by Slavin *et al.* (β-thalassemia major, Fanconi's anemia, Blackfan Diamond anemia and Gaucher's disease).[14] In a separate report[35] the Hadassah University Hospital transplant group reported the case of a child with Fanconi's anemia and leukemic transformation who underwent successful transplantation following a non-myeloablative conditioning regimen consisting of fludarabine, cyclophosphamide and ATG. Two patients with primary T-cell immunodeficiency have been described who received only post-transplant immunosuppression with MMF and CYA.[37] Stable multilineage mixed chimerism was seen in both patients. Both patients developed grade II acute GvHD that responded to prednisone therapy. Studies of immune reconstitution in one patient showed a significant increase in the numbers of T-cells, T-cell subsets and T-cell proliferative responses *in vitro*.

Given the therapeutic dilemmas that have surrounded the application of allogeneic BMT for conditions such as sickle cell anemia and thalassemia major, particularly the early transplant-related mortality risk among a group of patients who may have prolonged survival with medical therapy alone, these non-myeloablative transplant approaches may have particular benefit. The advantages include a low risk of transplant-related mortality, the development of stable mixed erythroid chimerism and the lack of a need to enhance a GvL effect by giving delayed DLI, all of which make this strategy particularly attractive for non-malignant disorders.

Remaining questions and future directions

The feasibility of achieving a state of stable mixed lymphohematopoietic chimerism, even following transplants from HLA-mismatched donors, and the use of this mixed chimerism as a platform for subsequent adoptive cellular immunotherapy, are the first important questions regarding non-myeloablative allogeneic stem cell transplant strategies answered by several centers. However, it is likely that just as many questions remain regarding the utility of these approaches as have been answered. The optimal non-myeloablative regimen has not been determined and it is unlikely that a single conditioning regimen will prove to be superior to others or applicable to all situations. Rapidly progressing hematologic malignancies will, in most situations, require initial cytoreduction of the tumor (thus probably requiring a reasonably aggressive chemotherapeutic preparation) in order to test whether a later GvL effect (such as, for example, that induced or potentiated by later DLI) will be operative. On the other hand, indolent hematologic malignancies (for instance, early CLL) or non-malignant disease may be optimally managed with conditioning regimens of lesser intensity. Long-term follow-up will also be required to determine the toxicity of these regimens.

Non-myeloablative HSCTs appear to be associated with significantly less transplant-related morbidity, and possibly mortality.[14,20-22,24-30] The incidence of acute GvHD still represents an important issue. The prophylaxis of this complication as well as its duration need to be studied and defined. Furthermore mixed chimerism may create an important platform for the administration of adoptive cellular immunotherapy (DLI) and for the optimization of the GvL effect; however this procedure is still too toxic and non-specific. The identification of tumor antigens for donor-vaccination or adoptive transfer of tumor-specific cytotoxic T-lymphocytes will, in the future, represent possible ways for reducing these limitations.[37] Regarding the optimal source of stem cells, both bone marrow and growth factor-mobilized peripheral blood stem cells have been used. There is a suggestion that immunologic recovery is faster following transplants in which peripheral blood stem cells are used than in those in which bone marrow is grafted.[38,39] However, hematologic recovery is usually rapid following non-myeloablative preparative regimens, regardless of the stem cell source. Thus, a comparison of antitumor efficacy, when bone marrow or peripheral blood stem cells are used, needs to be performed. Theoretically, the markedly increased number of T-cells in a peripheral blood stem cell allograft could promote earlier and more complete donor chimerism and obviate the potential platform for delivering DLI.

The published series of non-myeloablative stem cell transplants have primarily involved patients with refractory hematologic malignancies or those who were otherwise poor candidates for conventional allogeneic HSCT. As questions about feasibility and safety were answered, testing this approach in patients with less advanced disease and prospective randomized trials will be necessary to determine safety and efficacy in other diseases such as multiple myeloma and CML. Further studies of the efficacy of non-myeloablative transplant strategies for HLA-mismatched donor transplantation will also be required. These studies will be important, both because of the frequent need for alternative donor sources and because of the enhanced antitumor (i.e., GvL) effect that may occur in the setting of HLA incompatibility.

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Hematopoietic stem cell transplantation in the treatment of pediatric acute lymphoblastic leukemia: a pediatric single center experience

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The outcome of children with acute lymphoblastic leukemia (ALL) has improved dramatically since the introduction of aggressive front-line chemotherapy.[1,2] Nevertheless, 25% of children experience a relapse in bone marrow (95%) or in isolated extramedullary sites (5%) and are candidates for salvage therapy.[3] Several studies have shown that allogeneic (allo) hematopoietic stem cell transplantation (HSCT) from an HLA identical sibling offers a high probability of rescuing these children and their disease-free survival (DFS) is superior to that obtained by either a second course of chemotherapy or autologous (auto) HSCT.[4]

We retrospectively reviewed the outcome of 60 consecutive children affected by ALL who underwent either allo or auto HSCT in 2nd complete remission (CR) at our Institution from 1985 until January 2002.

Materials and Methods

From January 1985 to January 2002, 60 consecutive children affected by ALL in 2nd CR underwent HSCT at our Institution. The front-line treatment was that laid out in the AIEOP treatment protocols of the 79,85,87 series or 88-95 BFM generations,[1,2] and included intrathecal therapy and/or cranial prophylactic irradiation. Over the years, patients lacking a matched family donor (MFD) were switched from autologous transplant to matched unrelated donor (MUD) transplantation because of the encouraging results obtained in this latter setting.[5] Patients with bone marrow relapse received either auto-HSCT or allo-HSCT and were conditioned with total body irradiation (TBI) 12 Gy, and vincristine 5 mg/m^2 continuous infusion, cyclophosphamide 120 mg/kg[6,7] or thiothepa 5-10 mg/kg, cyclophosphamide 120 mg/kg.[8] Cyclosporine alone in MFD transplants or cyclosporine and short-term methotrexate and anti-lymphocyte globulin (3.5 mg/kg from day –4 to –2) in MUD transplants were used as graft-versus-host disease (GVHD) prophylaxis. Eighteen patients in the autologous series received TBI 14.4 Gy in 12 fractions and aracytin 24 g/m^2 after an isolated extramedullary relapse.[9]

AlloHSCT

Thirty-two children at the median age of 9.56 (range 2.57-19.10) years underwent allo HSCT in 2nd CR, 16 from an HLA MFD (BM = n.14; CB = n. 2) and 16 from a MUD, all (except 2 CNS relapse = 1MFD, 1 MUD) after a bone marrow relapse. The median duration of 1st CR was 32 (range 4-51) and 38 (range 18-53) months for the group infused with a MFD and MUD transplant, respectively. In the matched family group the donor/recipient pairs were all HLA matched while in the unrelated series 5 out 16 pairs were 1 HLA antigen mismatched at high resolution level (class I = n. 1; class II = n. 4). A median of 3.6×10^8/kg (range 1.5-4.4) TNC and a median of 4.8×10^8/kg (range 2-7.6) were infused to patients receiving MFD or MUD transplant, respectively. Two patients transplanted with cord blood received 2.9×10^7/kg and 6×10^7/kg TNC, respectively.

AutoHSCT

Twenty-eight children were given an auto-HSCT after an isolated bone marrow (n. = 10), an isolated CNS (n. = 10) or testes (n. = 8) relapse. The median age at transplant was 8.38 (range 3.32-16.76) years. The median duration of 1st CR was 32.5 (range 21-37), 27 (range 14–94), 23 (range 12–34) months, respectively after a bone marrow, CNS, and testes relapse. In 14 out of 28 patients the marrow was purged with maphosfamide at

a standard dose of 100 mg/mL;[7] 4 patients received a cycle of interleukin-2 in i.v. continuous infusion before the bone marrow harvest.[10] A median of 1.95 (range 1.1-4.6) $\times 10^8$/kg TNC were infused. Three patients received peripheral blood stem cells containing at least 4×10^6/kg $CD34^+$ cells.

Results

The 6-year disease-free survival of the whole group was 60.4% and the plateau was reached at 4 years after the transplant (Figure 1).

AlloHSCT

All patients engrafted without significant difference between MFD and MUD recipients. The polymorphonuclear cell count exceeded 500/mm^3 after a median time of 15 (9-18) days and the platelet count was > 50×10^9/L after a median time of 30 (range 15-60) days. Twenty-one out of 32 patients are alive and well. A comparable number of events were observed after either MFD (n. = 6) or MUD transplant (n. = 5) leading to a 6-year event-free survical of 53.5% (CI 23.3 –83.7) for MFD transplants and 66.6% (CI 42.5-90.7) for MUD transplants (Figure 2). The transplant-related mortality (TRM) was identical consisting in 1 death in each group, 39 days after BMT in the recipient of a matched family transplant and at 67 days in the patient who received a graft from a matched unrelated donor. The relapse rate in the MFD group was 35.7% (CI 10.6-60.8), compared to 17.5% (CI 0-39.6) in the MUD series. Twenty-eight out of 32 patients experienced acute GVHD; 20 children had grade I-II disease, while the other 8 developed grade III–IV disease. Ten out 20 patients, 5 with grade I-II and 5 with grade III-IV disease died of acute GVHD. The 6-year event-free survival according to the grade of acute GVHD was 74.6% (CI 55.4-93.8) for those with I-II grade disease compared to 37.5% (CI 39.5-71) for the group with grade III–IV acute GVHD, respectively. Nine patients (MFD= 5, events= 2; MUD=4, events=0) developed chronic GVHD leading to a 6-year EFS in this group of 77.7% compared to 46.8% in the series of 23 patients without chronic GVHD.

AutoHSCT

All patients showed durable engraftment of WBC and platelets. The WBC engraftment was observed after a median time of 18 (range 15-21) days and 14 (range 12 –18) days for purged or unpurged bone marrow, respectively. Platelet engraftment was seen after a median time of 35 (range 30–50) days and 25 (range 20–45) days for purged or unpurged bone marrow, respectively.

The survival of patients transplanted after a bone marrow relapse was very poor because only 1 out 10 such children is alive and well 10 years after transplant. In contrast, only 4 deaths (3 relapses and 1 infection) were observed in the group of 18 patients with an isolated extramedullary relapse leading to an event-free survival of 76.3% (CI 55.9-96.8) as shown in Figure 3. In particular, no deaths were observed in the small group of patients transplanted for testes relapse. Consequently, the relapse rate was 90% and 19.12% in patients transplanted for bone marrow or isolated extramedullary relapse, respectively. In the whole group the TRM was 5.5%.

Discussion

In this paper we report our experience in 60 consecutive children with ALL who underwent HSCT in 2nd CR in our BMT unit. In our experience the TRM in both the MFD and MUD groups was very low being 6.5%, according to the trend already

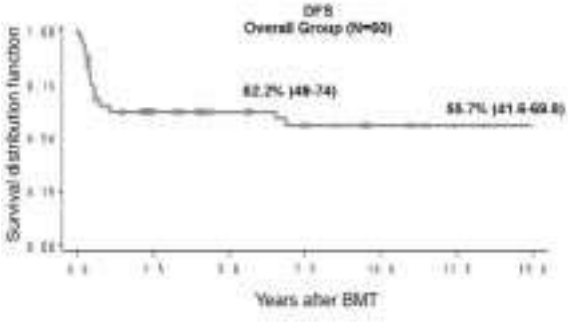

Figure 1

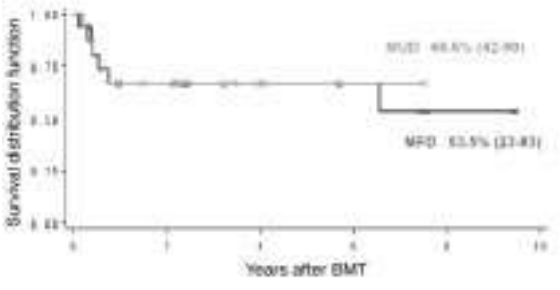

Figure 2. EFS of patients receiving MFD or MUD.

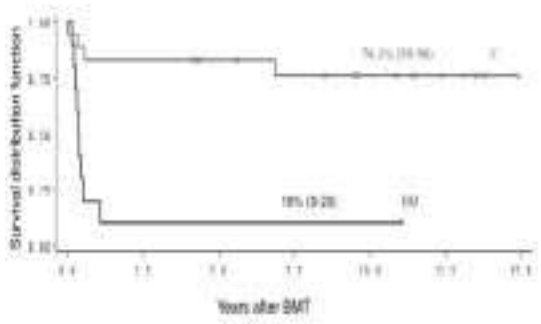

Figure 3. Auto-HSCT: EFS of patients according to the site of relapse.

observed in the last years by the AIEOP BMT group, in a larger number of patients.[11] It is important to note that this TRM rate is quite similar to that observed in the auto-HSCT recipients, i.e. 5.5%. These results are probably a consequence of the improvement in GVHD prophylaxis and therapy, the new techniques for the diagnosis of infections and the introduction of new active antibiotics and antiviral agents. Moreover, the relapse rate was higher in the MFD group than in the MUD group (35.7% versus 17.5%) suggesting a possible role of the unrelated donor immune system in controlling minimal residual disease. All these considerations lead us to extend the use of MUD transplants to patients who need an alloHSCT but lack a suitable familiar donor, given the poor results that have been obtained with autoHSCT after a bone marrow relapse, independently of the duration of 1st CR.[12]

The role of autoHSCT in the treatment of isolated extramedullary relapse is still controversial. In the past we reported encouraging event-free survival in patients who underwent autologous HSCT after an isolated extramedullary relapse.[7,9,12,13] In addition to our previous data, this report shows that auto HSCT is a good choice also for patients with testicular relapse.[14-18] Moreover, these data are comparable to the AIEOP results on patients who underwent auto HSCT or CT after a CNS relapse in 2nd CR which showed that the transplanted group fared significantly better than the group treated with chemotherapy (event-free survival: 56.3% versus 12.6).[12]

In conclusion, in accordance with national and international experiences, our data indicate that MUD transplant is the best choice for all ALL patients lacking a suitable familiar donor, even if they are at intermediate risk. We also suggest a positive role of auto transplant in the treatment of isolated extramedullary relapse.

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Historical perspectives, rationale and future directions for hematopoietic stem cell transplantation for severe autoimmune diseases

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Autoimmune diseases have been defined as a fascinating but still poorly understood group of diseases,[1] which pose *some of the most baffling scientific questions and daunting clinical challenges in internal medicine*.[2] This intricate and still imperfectly elucidated background must be considered before reviewing the history and, even more so, the rationale of hematopoietic stem cell (HSC) therapy for severe autoimmune diseases. Their etiology is clearly multifactorial, as reflected in the concept of an integrated fabric of components known as the *mosaic of autoimmunity*.[3] A first distinction must be made between autoimmune conditions produced by lymphoproliferative diseases which may be overt but also occult (autoimmune monoclonal gammopathy of unknown origin) and include paraproteinemic (IgM) polyneuropathy and chronic cold agglutinin disease (CAD), in which autoantibodies display a monoclonal pattern (monoclonal autoimmunity[4]), and for which successful HSCT may be clearly curative, and the more common organ-specific and systemic autoimmune diseases, which generally follow the pattern of antigen-driven immune reactions.[5,6] In general, however, both immune stimulation by infections[7] and molecular mimicry[8] mechanisms play important roles. When considering the interaction among genetic and non-genetic factors it must be kept in mind that the concordance rate for identical twins in most autoimmune diseases are between 15% and 30%[9,10] with a genetic risk, in the case of SLE, of 20 for siblings and 250 for monozygotic twins.[11] The availability of multiple murine models of systemic lupus erythematosus (SLE) has been pivotal for understanding many genetic and environmental factors.[12] Thus far, at least 45 named loci have been reported to be linked with one or more lupus traits in murine lupus,[13] while the presence of susceptibility genes within several chromosomal regions has also been confirmed in humans.[14] Recent studies of genetic reconstitution with polycongenic murine strains have characterized three susceptibility genes,[15] of which SLE 1 mediates the loss of tolerance to chromatin, SLE 2 lowers the activation threshold of B-cells, and SLE 3 mediates a dysregulation of $CD4^+$ T-cells, which in human patients have been shown to display features of a secondary antigen driven immune response.[16] For most primary severe autoimmune diseases multiple genetic, environmental, and hormonal factors conspire to instigate the etiopathogenesis.[17]

Experimental studies

Animal models of autoimmunity are important for our understanding and treatment of human autoimmune diseases,[18] and it is typical that stem cell transplantation (SCT) therapy started with mouse experiments. The history of these experimental studies is quite complicated, and a simplified overview may be helpful (Table 1).

A wide spectrum of experimental autoimmune diseases were cured following allo-bone marrow transplantation, including murine lupus, autoimmune thrombocytopenic purpura, crescentic glomerulonephritis and others. In MRL/lpr mice, which relapsed after conventional BMT, the integration with donor stromal cells was found to be curative.[19] A scarcely cited but interesting finding was the demonstration that human cord blood HSC were capable of suppressing autoantibody production in lupus mice,[20] perhaps the first example of a curative effect by xenogeneic stem cells.

Graft-versus-autoimmunity

One of the most important findings in allogeneic BMT for leukemic and other malignancies, both in animal experiments and in human disease, is the well-known graft-vs-leukemia (GvL) effect. As stated recently, this GvL effect *is very real, and thousands of patients are alive because of it*.[21] Evidence is now accumulating both in experimental and clinical settings, that a similar graft-vs-

Table 1. An overview of SCT studies for experimental autoimmune diseases.

* First transfer of murine SLE following marrow/spleen allo-BMT (1969-1974)
* Cure of murine SLE following allo-BMT from healthy mice
* Identification of disease-information carriers in HSCT
* New strategies utilizing donor stromal cells, osteoblasts and portal and intraosseous accesses
* Demonstration of a graft-versus-autoimmunity effect
* Utilization of non-myeloablative conditioning regimens
* Positive results following autologous SCT

Table 2. Main features of the history of SCT for clinical SADs.

* Adoptive post-transplantation autoimmunity
* Cure of coincidental diseases following allo-BMT
* Clinical studies with autologous SCT
* Phase I/II and inception of phase III studies
* First results with non-myeloablative allo-BMT
* Complete remissions following-intense immunosuppression alone

autoimmunity (GvA) effect might also exist, consisting in the substitution of normal T-, B- and lymphoid progenitor cells in the place of an autoimmune lymphoid system.[22] This GvA effect is supported by experiments showing that mixed chimerism obtained utilizing a sublethal irradiation conditioning regimen followed by allogeneic BMT can prevent the onset of diabetes and even reverse pre-existing autoimmune insulitis in non-obese diabetic (NOD) mice, whereas the same radiation protocol without allogeneic SCT is insufficient.[23] A similar effect has been shown using sublethal conditioning and an anti-CD154 monoclonal antibody.[24] These findings are being currently reproduced in the clinic.

Autologous SCT

The apparently paradoxical idea of curing autoimmune diseases utilizing the patient's own stem cells following irradiation protocols originates from the provocative animal experiments pioneered by Dirk van Bekkum and his group.[25] First they demonstrated that autologous BMT was capable of curing adjuvant arthritis in rats,[26] and then that closely superimposable results could also be obtained in experimental allergic (autoimmune) encephalomyelitis (EAE),[27] in which however relapses could be prevented only following allogeneic transplants. These results ignited interest and widespread trials with autologous procedures in clinical autoimmunity. Extensive reviews of this area have been published.[19,28]

Clinical results

The history of SCT transplantation for clinical severe autoimmune diseases is also quite complicated; the main features are shown in Table 2.

This is an extremely active and investigated area; recent extensive reviews[29,30] are requested readings for anybody wishing to engage in it. In particular two exhaustive reviews have been published in this same journal.[31,32]

Conclusions and future directions

There are now three new aggressive approaches for the treatment of severe autoimmune diseases of the refractory (relapsing) life-threatening subtype. High-dose cyclophosphamide with no stem cell rescue has had encouraging results in the John Hopkins single center experience.[33] However intense immunosuppression is most generally followed by infusion of hematopoietic stem and progenitor cells included in the CD34 selected compartment. Autologous HSCT, which originated from the classical animal experiments by van Bekkum[25] and Ikehara[19] are being utilized worldwide because of the procedure's greater safety, although transplant-related mortality (TRM) has been unexpectedly high.[34] Patients with multiple organ insufficiency because of the ubiquitous damage inflicted by advanced systemic autoimmune diseases appear to be at greatest risk of TRM. The more intense immune suppressive preparative regimens when combined with $CD34^+$ selection of the graft have also been associated with fatal opportunistic infections.[35] It is still uncertain whether the mechanism of action is essentially immunosuppressive, or whether lymphoid reconstitution following mobilization plus conditioning may ensure the emergence of a tolerant immune system *vis-à-vis* of the same autoantigens that had driven the autoimmune process.[36,37] Be this as it may, clinical results are encouraging and even dramatic in properly selected patients. Some of the best results are being obtained in SLE as discussed elsewhere[22] and in active progressive multiple sclerosis, in which abrogation of all gadolinium enhancing lesions has been found in Genoa.[38] From the initial phase I/II clinical studies, randomized phase III trials are currently evolving in Europe, including the ASTIS (systemic sclerosis), ASTIMS (multiple sclerosis) and ASTIRA (rheumatoid arthritis) trials and in America as NIH-funded trials for multiple sclerosis, SLE, and scleroderma. The utilization of allogeneic HSCT has obviously a greater biological appeal. HLA identical stem cells may carry identical susceptibility genes; however complete remissions have been reported following syngene-

ic transplants in cases of severe rheumatoid arthritis[39] and of chronic refractory autoimmune thrombocytopenic purpura.[40] A prolonged follow-up of these cases might offer information on the role of autoantigenic rechallenge. Non-myeloablative allogeneic transplants (NST) are attractive not only because of their limiting effect on TRM,[41,42] but also because a GvA effect has been demonstrated in experimental autoimmune diseases, and seems to be present also in humans.[43] Relapse of coincidental autoimmune diseases was shown to be more frequent in patients without GvHD.[44] This is reminiscent of the very first observations in leukemia, in which the immunotherapeutic effect of allogeneic HSCT is most evident.[45] In addition, there are now two well-documented case reports of non-myeloablative allogeneic transplants (NST) for Evans' syndrome in which complete clinical and immunologic remissions appeared following GvHD, in one case elicited by donor lymphocyte infusions (DLI).[46,47] However separating GvH from GvA appears as hard to obtain as GvH from GvL. Superimposing GvH to a patient with a severe autoimmune disease is not a good proposition, but to harness GvA in order to eradicate the last autoimmune lymphoid clones would seem a reasonable objective in the not-too-distant future. Will this be all that it takes to cure autoimmune diseases, which are a combination of pathogenetic immune autoreactivity and multiple (auto) antigenic challenges? The answer to this fundamental question will be found only by a continuous and hopefully fruitful cooperation between basic and clinical investigators.

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Legislative and ethical aspects of administering granulocyte colony-stimulating factor to normal donors

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The use of conditioning agents in normal donors to improve the collection of blood components has been long practised. For instance, when considering the donation of whole blood, particularly in females, supplementation of ferrous sulphate to restore iron deposits following blood collection is commonly recommended. Moreover, glucocorticoids (prednisone/dexamethasone) have usually been given to normal donors undergoing leukapheresis for granulocyte collection. Such a practice is substantially well-accepted, according to some provisions of national law on blood transfusion, recently promulgated in Italy.[1] As a matter of fact, administration of ferrous sulphate and glucocorticoids does raise important issues of safety, since both drugs are characterized by several troublesome side effects, including insomnia, palpitations, flushing, euphoria, and upper/lower gastrointestinal complaints. At present, Italian legislation does not provide for the donor's informed consent when these drugs are administered, as clinical experience probably conforms with the chief principle of blood transfusion practice, namely *primum non nocere* (non-maleficence). In other words, transfusion medicine physicians should aim at minimizing, as much as possible, any risk to the blood donor's health, while at the same time assuring the greatest benefit to their patients.

All these considerations, we believe, cannot be fully complied with when using granulocyte colony-stimulating factor (G-CSF) in healthy normal donors. The pharmacologic use of this cytokine in normal donors was introduced in the early 1990s,[2] both to increase the yield of granulocyte collection[3] and to mobilize peripheral blood stem cells (PBSCs) for allogeneic transplantation.[4-6] Granulocyte transfusion as a supportive treatment of neutropenic patients had been widely used until 1985. Thereafter, the use of this therapeutic option underwent a dramatic decline, owing to the conflicting results of some clinical studies. In fact, only partial benefit from granulocyte transfusion had been demonstrated, conflicting with the high frequency of severe adverse effects, including pulmonary distress and cytomegalovirus infection, in recipients.[7,8] The lack of recovery following granulocyte transfusion in patients with persistent neutropenia, along with clinically or microbiologically defined infection, could be due to the rather low cell yield of granulocyte concentrates, if it assumed that the neutrophil dose is the main determinant of clinical efficacy of this therapeutic tool. On this subject, it should be noted that the turnover of neutrophils in normal adults is very high, i.e., approximately 60×10^9 cells/kg/day and may be increased several fold when severe bacterial infections occur.[3]

The introduction of high efficiency apheresis technologies and the approved pharmaceutical forms of recombinant G-CSF for human use in the last decade has renewed interest in granulocyte transfusion. Indications for granulocyte transfusion, however, are currently strictly defined. Generally accepted criteria for such a treatment are severe neutropenia with polymorphonuclear cells $<0.1\times10^9$/L, at least for 7 days, resistance to pharmacotherapy, including G-CSF, microbiologically and/or clinically defined infection.[9] Recent data, albeit encouraging, indicate the need for further clinical trials to validate the clinical efficiency of granulocyte transfusion.[10-13] At present, G-CSF administration to normal donors is not approved by health authorities, since little if any evidence actually exists to assure the long-term safety of

this product, in particular concerning the putative risk of triggering hematologic malignancies. The short-term toxicity induced by G-CSF administration is already well recognized. Adverse effects are generally self-limiting, resolving in all cases within a week following the last dose. Nevertheless, they are very frequent and troublesome, so that this issue cannot be disregarded when normal donors are considered. According to the information sheets of some commercially available G-CSF products, the most common drug-related side effects are headache (40%), bone pain (23%), fatigue (11%), abdominal pain (6%), diffuse joint pain and myalgia (6%). Moreover, transient increases of AST and ALT have been observed in, respectively, 12% and 16% of donors.

As a matter of fact, G-CSF has been used in normal donors as a priming agent to mobilize PBSCs for allogeneic transplantation, but strictly as a part of study protocol, requiring informed consent.[5,6] In fact, PBSC collection from unrelated healthy donors by leukapheresis clearly has certain advantages over standard bone marrow donation in that it avoids general anesthesia and reduces time spent in hospital, since the post-donation clinical and hematologic recovery is very brief.[14-16] Moreover, when considering the effect on the recipient, the antileukemic potential of PBSC allografts has been reported to be greater than that of bone marrow,[17] although this issue is still being debated.[18]

Use of G-CSF and legislative standards on transfusion practice

The legislation on transfusion practice that has been recently promulgated in Italy does not refer, in any part, to the use of G-CSF or other hematopoietic growth factors in healthy blood donors (*see Appendix 1*). When debating donation by apheresis, in generic terms *a possible drug premedication* is mentioned. In another subsection, this concept is again considered as a practice *allowed exclusively in few cases, fully justified, following consent of donors that should be carefully informed of all details of the procedure*. However, afterwards when the legislative text deals with the collection of PBSCs, normal donors are reported as a source of allografting. Obviously, this type of collection is possible only by conditioning donors with hematopoietic growth factors, but the law does not provide for this practice. In another article concerning the donation of PBSCs, it is asserted that *different criteria of idoneity may be accepted, on the condition that the donor's health status must be absolutely protected*. The conclusion thus appears to be that it is up to the transfusion medicine physician, after all, to decide, with complete understanding and acceptance of his liability.

Thus, Italian legislation on transfusion practice does not indicate clear-cut criteria for the administration of hematopoietic growth factors to normal donors and neither has the Council of Europe, according to the *Guide to the preparation, use and quality assurance of blood components, 6th edition, 2000* (*Appendix 2*) provided definitive indications. Indeed, premedication with glucocorticoids and G-CSF is strongly discouraged until further detailed data on the safety of such drugs in normal donors are obtained. With regard to the PBSC collections, all pretreatment conditioning to increase the yield of collection should be prescribed in conformity with professional codes of conduct, after donors have given informed consent in writing.

G-CSF related adverse effects

The use of G-CSF is associated with a number of adverse effects. Nevertheless, toxicity is generally mild and only in few cases requires discontinuation of the G-CSF. According to the results of a study based on a multivariate analysis,[19] a correlation between donor's sex, G-CSF dosage and certain adverse effects has been found. In particular, G-CSF at doses greater than 8 μg/kg/day more frequently induced bone pain, whereas headache was more common in donors younger than 35 years old. Gastrointestinal complaints (nausea, vomiting) were recorded mostly in female donors. From an overview of some literature data,[20] concerning 672 normal donors undergoing G-CSF administration, 486 (72.3%) suffered from bone pain, 163 (24.2%) from headache, 71 (10.6%) from severe fatigue, 68 (10.1%) from insomnia, 26 (3.9%) from fever, 23 (3.4%) from nausea, and 10 (1.5%) from myalgia and anorexia.

Severe adverse effects have been very seldom reported. In 1999 Falzetti *et al.*[21] described spontaneous rupture of the spleen in the course of PBSC mobilization: on the 5th day of G-CSF the donor complained of pain below the left costal margin and during the following night showed signs of acute anemia and shock. He underwent an emergency splenectomy and was discharged two weeks after surgery. A similar case had been previously reported by Becker *et al.* in 1997.[22] The risk of such a life-threatening, albeit rare, complication has been recently stressed by Platzbecker *et al.*,[23] who demonstrated a slight, but significant spleen enlargement in healthy donors during mobilization of allogeneic PBSCs by G-CSF.

Moreover, Parkalli *et al.*[24] reported a case of acute iritis occurring in a voluntary unrelated PBSC donor, who had a history of abdominal pain and skin rash due to a mild form of, respectively, coeliac disease and dermatitis herpetiformis. This complication, ascribable to the G-CSF administration, involved a rapid sequence both eyes and resolved within a few days following local corticosteroid and scopolamine treatment.

According to a report by Anderlini *et al.*,[25] a 54-year old woman had a cerebrovascular accident two days after completing an uneventful PBSC donation, in the absence of any risk factor of thrombosis. Even though the relationship between the procedure of PBSC collection, including G-CSF administration, and this event seems to be uncertain, the chronology should raise suspicion of a possible link.

As opposed to the short-term toxicity, which is well documented and, on the whole, mild, the major concern about G-CSF administration to normal donors is the putative risk of long-term adverse effects. In fact, it cannot be excluded that such premedication might stimulate an abnormal clone, thus triggering an overt hematologic disorder. In this regard, Freedman *et al.* observed an overall onset of myelodysplastic syndrome/acute myeloid leukemia in 31 (9%) of 352 patients affected by congenital neutropenia following G-CSF therapy, after a median follow-up of 6 years.[26] As a matter of fact, such disease transformation could be attributed to the risk associated with the primary hematologic abnormality rather than to the G-CSF exposure. A recent analysis of data from the *Severe Chronic Neutropenia International Registry* (SCNIR), including patients who had received G-CSF for up to 11 years, did not identify any adverse effects associated with prolonged duration of treatment.[27]

Moreover, Cavallaro *et al.*[28] studied the effects of G-CSF in 101 normal donors undergoing PBSC or granulocyte collection. After a median follow-up of 40 months, no increase in drug-related hematologic malignancies or other life-threatening complications were recorded. These authors concluded that the administration of G-CSF to normal donors appears to be safe and free of severe side effects. It has to be underlined, however, that the available data are not at present sufficient to exclude a potential, albeit unlikely, risk of hematologic malignancy, since it will require a database of 2,000 donors followed-up for at least 10 years to detect a 10-fold increase in the incidence of leukemia, according to the biometrical considerations of Hasenclever and Sextro.[29]

Ethical aspects of G-CSF administration to normal donors

Given the quantity of short-term adverse effects and the putative long-term risk of G-CSF, in the absence of any clear-cut provision of the law, administration of such a cytokine to normal donors has to be considered from an ethical point of view and in the context of a professional code of conduct.[30,31]

Ethical issues applied to the medical domain and public health comprise different stages including (a) research ethics, intended as the planning and the conduct of clinical trials; (b) ethics of the application of medical innovations to human beings (bioethics); (c) public health ethics, concerning the equal distribution of resources.[32]

According to definite recommendations of the Council of Europe (*Guide to the preparation, use and quality assurance of blood components*, 6th edition, 2000), blood donation should be voluntary and non-remunerated. Obviously, these principles accord with the guidance of the WHO and have been fully recapitulated by Italian law in the field of transfusion practice.

Voluntary, unpaid blood donation protects donors from psychological and/or material pressure to donate, thus assuring a total autonomy, right of self-determination and freedom of choice. Moreover, transfusion practices must, on all occasions, presuppose the principle of *primum non nocere*, by safeguarding the donor's health. Therefore, administration of drugs to produce or enhance the collection of certain blood components must be cautiously considered before being incorporated into routine use or even research protocols.

As regards the use of G-CSF, the putative risk for donors should be related to the possible benefit to recipients. On this issue, which cannot be ignored, there are two different situations: I) G-CSF as a conditioning agent for granulocyte collection. Since the benefit of granulocyte transfusion cannot be considered to be proven, since clear results of controlled clinical trials are lacking, this therapeutic practice cannot be claimed to be a unique life-saving tool; II) G-CSF as conditioning agent for PBSC mobilization. The administration of hematopoietic growth factors is an alternative option to traditional bone marrow harvesting, whose long-term safety should documented, at least on an average follow-up of 40 months.[33] As opposed to granulocyte donation, in both cases (PBSCs and bone marrow), the collection of progenitor cells is almost always the only therapeutic chance for recipients. Thus, in theory transfusion medicine

physicians, in accordance with all medical staff who are their joint partners in the planning of a bone marrow transplantation, should offer all knowledge to donors. Under these conditions, the donor could make a substantially free and voluntary choice between PBSC and bone marrow harvesting, after informed consent in writing. As pointed out by Volk *et al.*, Blood Transfusion Services and their professional staff have a responsability to provide an objective evaluation of risks and dangers associated with both kinds of donation (PBSCs and bone marrow).

The same approach should be used for related donors, with a clear disclosure of information. As a rule, kinship itself can be a source of strong psychological pressure, in some cases being an adjunctive risk to recipients. Indeed, directed blood donation from a family member is generally discouraged by transfusion specialists, in that donors might hide confidential medical information. It must be stressed that, when related donors are involved, informed consent, obtained as a merely formal action, may be not sufficient from an ethical point of view. In our opinion, only research protocols, reviewed and overseen by institutional committees, will be able to establish the use of G-CSF in normal donors, and in any case clinical trials must respect ethical principles.

In the context of these comments, stem cell mobilization of subjects of pediatric age (in some cases < 1 year) or older donors (> 70 years), or of individuals with previous neoplastic, cardiovascular, autoimmune disease, and the insertion of central venous catheters, exposing subjects to serious risks (e.g., pneumothorax),[6] should be very cautiously considered, if not excluded *a priori*, even within the setting of clinical trials.

Protocols of G-CSF administration to normal donors

A brief overview of the literature data shows that dosage and schedules of the G-CSF administration to volunteer donors are varied, but all aim at the most efficient yield of collection.[34-39] As previously pointed out, the primary aim of transfusion medicine physicians must be to safeguard the donor's health. In this regard, it would be desirable to introduce operative protocols on G-CSF use produced by the scientific community. Indeed, this approach, when systematically applied, could defend physicians in cases of legal litigation, by avoiding personal, and in several cases difficult, choices.

In what context should such operative protocols be implemented?

(I) a local session, consulting the Hospital Committee for Good Transfusion Practice, along with the Ethical Committee, in the active presence of all physicians involved in the donation procedure. The donor's family doctor will be fully informed, as primarily responsible for the post-donation follow-up;

(II) by means of *ad hoc* study groups, involving Blood Transfusion Services plus Hematology and Bone Marrow Transplantation Centers;

(III) by charging the Scientific Societies with the task of issuing guidelines that should inform subsequent legislative revision.

On this subject, the *Italian Society of Transfusion Medicine and Immunohematology* (SIMTI) has recently delivered some guidelines on the collection of progenitor cells for allogeneic transplantation,[40] by considering both bone marrow and PBSCs (in the latter case, by conditioning donors with G-CSF). When a PBSC collection is established, operative guidelines provide that:

1) the donor's suitability has to be ascertained, in full autonomy, following a complete clinical and laboratory evaluation, taking particular care to avoid any psychological pressure involving the donor and Bone Marrow Transplantation staff. The Apheresis specialist is responsible for this;[41]

2) the mobilization program must be arranged by the Apheresis and or the Bone Marrow Transplantation specialists;

3) the donor's follow-up, to monitoring late-onset toxicity due to G-CSF, is the responsibility of the physicians who administered the drug.

According to this protocol, which has been approved by relevant scientific societies, study groups and institutions, including the *Italian Society of Hematology* (SIE), the *Italian Society of Hemapheresis* (SidE), the *Italian Group for Bone Marrow Transplantation* (GITMO)[42] and the *Italian Bone Marrow Donor Registry* (IBMDR),[43] unrelated donors, at present, cannot undergo PBSC collection as a first donation. Besides these generic issues, when the donor's selection is considered, by differentiating familial and unrelated individuals, the problem of G-CSF administration to healthy volunteers remains at present unsolved, by referring to the recommendations that the *Executive Committee* of the *World Marrow Donor Association* had delivered in 1992.[44]

As a further example, a protocol from the National Marrow Institute entitled *Use of G-CSF*

mobilized leukapheresis collection from normal volunteers to develop improved methods of stem cells and lymphocyte selection for allogeneic transplantation has been approved by the US *Food and Drug Administration*.[45] According to this protocol, major eligibility criteria have been set as follows:

- normal healthy volunteer of either sex, between 18 and 60 years of age;
- no active infection or history of recurrent infection;
- normal liver, renal and cardiovascular function;
- subject must be eligible to normal blood donation (negative laboratory testing for transfusion-transmissible infections).

Donors can participate no more than 3 times; each time must be at least 3 months apart. Exclusion criteria are a history of autoimmune disease (e.g., rheumatoid arthritis and systemic lupus erythematosus), cancer (excluding squamous carcinoma of the skin) and hematologic disorders. Obviously, subjects must give informed consent to participate in the study.

Similar protocols, even adapted to certain clinical situations, that have been shared by the scientific community, should be divulged and applied, thus warranting both operative conformity and safety, and allowing larger data collection. Indeed, when considering the above mentioned protocol, up to April, 2000, more than 3,000 PBSC donors had entered the study.

We believe that the establishment of an International Registry of G-CSF-treated normal donors, both for granulocyte collection and PBSC mobilization, including experience from various countries, would allow sufficient information to be obtained to clarify, once and for all, the true long-term risks of G-CSF administration.

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Appendix. Italian legislation on transfusion practice

Gazzetta Ufficiale della Repubblica Italiana. Ministero della Sanità

D.M. 25.01.2001. Characteristics and conditions of blood component donation

Article 2. Collection by apheresis.

Paragraph 4. A possible donor's premedication, to enhance the yield of collection of certain blood components, may be allowed exclusively in few cases, fully justified, following consent of donor that should be carefully informed of all details of the procedure.

Article 5. Collection of peripheral stem cells.

Paragraph 2. Stem cells, that can be found in bone marrow, in cord blood and as a subpopulation of peripheral mononuclear cells, are drawn from healthy donors (allogeneic transplantation) or from

patients themselves (autologous transplantation). The requisite amount of cells to carry out transplantation must be determined on the basis of previously defined operative protocols.

Addendum 1. Leukocyte donation. Leukocyte donation by apheresis must be characterized by the following requisites:

a) collection of at least 1×10^{10} leukocytes in all per donation;

b) at the most 6 leukapheresis sessions per year, when the donor does not undergo premedication, are allowed; otherwise, when donor is treated with corticosteroids, the maximum number of donations per year is 4.

D.M. 26.01.200. Protocols for the assessment of the suitability of a blood component donor

Article 13. Donation of peripheral blood stem cells (PBSC).

Paragraph 1. Criteria for suitability of the PBSC donor are the same as those of a blood donor; moreover, risks relevant to the donation have to be weighed both by the transfusion medicine and bone marrow transplantation specialists (the latter, in the case of allogeneic donor).

Paragraph 2. In certain clinical situations, needing specific interventions, if necessary, different criteria for suitability can be adopted, according to the judgement of the transfusion medicine specialist, on the condition that the donor's health is fully protected.

Paragraph 3. The PBSC donor (for allogeneic and autologous transplantation) must be tested for laboratory markers of transfusion-transmissible infections at least 30 days before leukapheresis sessions

Appendix 2

Council of Europe
Guide to the preparation, use and quality assurance of blood components, 6th edition, 2000

Selection of donors

Donors of haematopoietic progenitor cells derived from bone marrow or peripheral blood.

Allogeneic donors of haematopoietic cells shall meet the health criteria established for normal whole blood donors.

In exceptional, life-saving situations, some deviation from the normal standards may be necessary, but in these situations the donor, the potential recipient, and their respective physicians should be informed and give their consent.

Donors shall be informed about the collection procedure and its potential risks. In the absence of sufficient data on the use of G-CSF (granulocyte colony-stimulating factor) in normal volunteer donors of peripheral blood progenitor cells such use should currently be limited to controlled trials.

Granulocytes: apheresis

Pretreatment of donors with corticosteroids and G-CSF is discouraged until the safety of such treatment has been further evaluated.

Haematopoietic progenitor cells

Methods of collection and preparation. All treatment of donors required to obtain an effective hematopoietic progenitor cell preparation should comply with the relevant medical ethics and be performed with informed consent of the donor.

Allogeneic transplantation. All requirements for donor selection and laboratory testing are applicable as for normal whole blood and cytapheresis donor next to full HLA typing. If the donor can not meet these criteria, deviation is permissible only after the documented approval of the donor's and recipient's physicians.

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The use of cytokine-stimulated healthy donors in allogeneic stem cell transplantation

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Treatment of healthy donors with recombinant human granulocyte colony-stimulating factor (rhG-CSF) allows the mobilization and peripheralization into circulating blood of an adequate number of CD34+ cells that can then be collected by leukapheresis (PBSC). This procedure avoids the invasiveness of bone marrow harvest and the risks related to general anesthesia. The main adverse effects of rhG-CSF are: bone pain, 84%, headache, 54%, fatigue, 31%, and nausea, 13%, which are usually scored by the donors as moderate to severe, resolving within 2-3 days after discontinuation of the cytokine. Analgesics, mainly acetaminophen, are sufficient to control the pain. Less than 5% of the donors experience non-cardiac chest pain, a local reaction at the injection site, insomnia, dizziness or a low-grade fever. Discontinuation of the PBSC procedure because of adverse effects of rhG-CSF or leukapheresis is rarely necessary (0.5%) but this good tolerability can be hampered by the need, in 5-20% of cases, for an adequate venous access that requires insertion of a central or venous catheter. There are no absolute contraindications to the stimulation of healthy donors with rhG-CSF but the description of cases of non-traumatic splenic rupture, iritis, cardiac ischemia, and gouty arthritis suggests that further precautionary restrictions are advisable when deciding eligibility for PBSC collection. The main advantages for patients receiving an allogeneic PBSC transplant are the faster hematologic and immunologic recovery and the potential for a greater efficacy in advanced disease by lowering the transplant-related mortality. One of the major concerns regarding the use of rhG-CSF in unrelated healthy donors is the uncertainty about its possible role in triggering malignancy, in particular myelodysplastic syndrome and acute myeloid leukemia. There are no studies with an adequate sample size and follow-up that can answer this question but two recent retrospective studies reported that in the medium term rhG-CSF is not associated with an excess of lymphoproliferative disorders. Currently, caution on the long-term safety of the use of rhG-CSF in healthy donor is still warranted but the data so far accumulated on allogeneic PBSC transplants are encouraging both as far as concerns the good short-medium tolerability profile of G-CSF-stimulation of the donor and the potential major efficacy in leukemia patients.

Key words: healthy donors, allogeneic stem cell transplantation, G-CSF.

Allogeneic hematopoietic stem cell transplantation is the best option of cure for a large number of patients with hematologic and non-hematologic diseases and the data from large registries show that this procedure is being increasingly performed.[1-3] The hematopoietic reconstitution after a myeloablative conditioning regimen is achieved by the administration of donor's repopulating progenitor cells, which are characterized by self-renewal potential, pluripotential properties and ability to initiate long-term bone marrow culture.[4] The presence of CD34 antigen on the cell surface identifies the primitive, non-lineage committed, progenitor cell population with stem cell characteristics.[5] At steady-state in healthy donors, the percentage of CD34+ cells is 1.1% of total nucleated cells in the bone marrow and as low as 0.06% among the circulating cells in the blood. This limited concentration of circulating CD34+, around 3-4/μL of peripheral blood, prevents collection of an adequate number of hematopoietic stem cells, suitable for a successful engraftment in allogeneic hematopoietic stem cell transplant, with single or multiple sequential leukaphereses in a short period of time.[4]

In the autograft setting, a transient peripheralization of CD34+ cells into circulating blood and an

increased yield of leukapheresis is obtained by *chemopriming* the patient with conventional high-dose chemotherapy followed by treatment with recombinant human granulocyte colony-stimulating factor (rhG-CSF), at a dose of 5-10 μg/kg/day, until the desired engraftment dose of $CD34^+$/kg cells has been collected. Chemopriming regimens are based on chemotherapeutic agents with low stem cell toxicity such as cyclophosphamide, ifosfamide, etoposide, and cisplatin.[6]

As far as allografting is concerned, several studies showed that treatment of healthy donors with rhG-CSF allows mobilization and peripheralization of an adequate number of $CD34^+$ cells, that is, an engraftment dose of at least $3\text{-}4\times10^6$ cells/kg body weight recipient, rendering this procedure a suitable alternative to harvesting bone marrow from the donor. A dose-response relationship has been demonstrated between the dose of rhG-CSF, up to 10 μg/kg/day, and mobilization of $CD34^+$ cells.[7,8] Higher doses have been explored but the experience is limited and a clear benefit in terms of cost-effectiveness has not so far been proved.[9] The administration of the glycosylated form of rhG-CSF (lenograstim) to healthy donors seems to be more effective at mobilizing $CD34^+$ progenitor cells than the non-glycosylated one (filgrastim).[7] The effect of rhG-CSF stimulation on peripheral increase of white blood cells (WBC), polymorphonuclear cells (PMN), lymphocytes and $CD34^+$ cells is quite uniform although there may be a large interindividual variability. Korbling *et al.* showed, in 41 healthy blood stem cell donors, that 3 days of rhG-CSF, at a dose of 12 μg/kg/day, caused increases of WBC, PMN, lymphocytes of 6.4, 8.0 and 2.2-fold over baseline, respectively.[10] An even higher concentration of peripheral blood $CD34^+$ cells was obtained, which increased 16.3 to 24.2-fold, respectively, depending on which subset was considered. Immunophenotype profile comparison between peripheral-rhG-CSF-stimulated and bone marrow $CD34^+$ cells showed that mobilization increases the percentage of myeloid precursors ($CD34^+$ $CD33^+$, $CD34^+$ $CD13^+$) and decreases B-lymphocyte precursors ($CD34^+$ $CD19^+$).[11]

The time to achieve a plateau in the mobilization of $CD34^+$ cells and other WBC subsets into peripheral blood is 4 or 5 days using the rhG-CSF at a dosage of 10-12 μg/kg/day and any longer stimulation may be associated with a decline in $CD34^+$ yield.[8] Considering 2 groups of HLA-matched related donors without significant difference in terms of age, weight, and apheresis blood volume processed, performing leukapheresis on day 5 more frequently resulted in the collection, with a single apheresis, of a target cell dose of 4×10^6 $CD34^+$cells/kg of recipient body weight than performing leukapheresis on day 4, but a trend to a higher, although not statistically significant so, concentration of $CD3^+$ lymphocytes was observed.[12]

Apart from the daily dose of rhG-CSF, the factors that may affect mobilization in healthy donors and the subsequent stem cell yield by leukapheresis have not been clearly defined yet. According to Anderlini *et al.*, the once-daily versus twice-daily schedule of rhG-CSF administration resulted to be similarly effective in mobilization and collection of $CD34^+$ progenitors cells in normal donors following stimulation with filgrastim at the cumulative dose of 12 μg/kg/day for 3 days, starting daily leukapheresis on day 4.[13] Conversely, the assessment of $CD34^+$ cell yield in 119 healthy donors who underwent leukapheresis on day 4-6, after stimulation with 12 μg/kg/day of filgrastim, showed that only age greater than 55 years was a significant risk factor in multivariate analysis for poor mobilization.[14] In order to boost the peripheral blood $CD34^+$ collection, combinations of rhG-CSF with other cytokines, such as recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF) or Flt3 ligand, have been investigated, albeit in limited studies. The efficiency of stem cell mobilization was not augmented by the addition of rhGM-CSF while Flt3 doubled the peak of $CD34^+$ cells compared to rhG-CSF alone.[15,16]

Cytokine-mobilized peripheral allogeneic stem cell transplantation: implications for donors and patients

Over the last two decades, bone marrow harvesting became a common practice in transplant centers but it still raises concerns about safety because of its invasiveness and need for general anesthesia. Acute post-bone marrow donation complications were reported to occur in as many as 5.9% of donors and required a mean prolongation of hospitalization of 2 weeks. Life-threatening complications were rare (0.27%) but 2 deaths among 7,857 donations (0.02%) were recently reported by the IBMTR for the period 1994-1998.[17]

Generally speaking, the collection of mobilized peripheral $CD34^+$ cells by leukapheresis is simpler and more convenient for the donor. It avoids the invasive procedure of bone marrow harvest by multiple bone aspirations under general or spinal anesthesia; moreover, the time of recovery is shortened and the procedure does not require hospitalization. The target $CD34^+$ cell dose is achieved with one leukapheresis in 60% of donors, with 2 leukaphere-

ses in another 30% of donors and with 3 leukapheresis in the remaining 10% of donors. The discontinuation of leukapheresis because of adverse effects is rarely necessary (0.5%) and is mostly associated with toxicity of rhG-CSF administration.[18] The good tolerability of the procedure may be hampered by the need for an adequate venous access that requires, in 5-20% of cases, insertion of a central venous catheter.[18,19] This may add risks to and reduce the acceptability of the procedure, require or prolong hospitalization and limit its application in children or in older donors. So far, there are no absolute contraindications to the use of rhG-CSF in healthy donors who meet the criteria for blood and platelet donation but the description of cases of non-traumatic splenic rupture, iritis, cardiac ischemia, and gouty arthritis suggest that some precautionary restrictions must be adopted before granting eligibility for the procedure. In particular, the potential risks of administration of rhG-CSF must be considered in donors with a history of ocular inflammatory diseases, autoimmune diseases, cardiovascular or thrombotic diseases or familial leukemia.[20]

As far as the patient is concerned, the clinical outcome of peripheral and bone marrow stem cell transplantations has been recently reviewed by Korbling *et al*.[21] So far, six randomized trials, one matched-pair study and one restrospective analysis from the IBMDR and EBMT Registries have been published; moreover, two other randomized studies have been presented in preliminary form at the 2000 Meeeting of the American Society of Hematology, one from the EBMT and one from the Canadian Bone Marrow Transplant Group; the conclusive results are awaiting.[22-29] All the studies reported a statistically faster recovery of neutrophils and platelets ranging from 12 to 19 days and 11 to 18 days, respectively following peripheral blood stem cell transplant. Other major differences have been reported regarding the remaining main outcomes after transplant, but the results from different studies are not always the same. A higher incidence of acute graft-versus-host disease (GvHD) after allogeneic PBSCT was described only in the preliminary data from the EBMT using a regimen of GvHD prophylaxis of 3 doses of methotrexate.[27] Chronic GvHD was significantly more common following PBSCT in 4 of the above-mentioned studies and has been associated with a higher absolute number of T-cells (approximately 1 log higher) infused in unmanipulated PBSCT. One hundred-day transplant-related mortality was significantly lower in PBSCT recipients in the study by Simpson *et al*.[28] (7.5% vs. 16%) and in that by Champlin *et al*.[29] In this latter study the difference was noted for the patients in more advanced disease, i.e., for patients transplanted in second complete remission of acute leukemia (13% vs 30%) and in accelerated phase or in second chronic phase of chronic myeloid leukemia (26% vs 67%). The relapse rate was comparable in all studies except that by Powles *et al*., who reported a lower 2-year probability of relapse in PBSCT versus BMT recipients: 0 vs 37%.[26] Significantly, a higher probability of 2-year disease-free survival has been noted in 3 studies, 2 randomized and one retrospective, notably because the transplant-related mortality was lower in patients with more advanced-stage disease.[28-30] Moreover, data from Ottinger *et al.* showed a faster immune reconstitution in the first year after transplant in favor of PBSC recipients. This is probably related to the higher number of $CD34^+$ infused or the administration to the patient of the donor's immunocompetent cells collected with the leukapheresis.[31]

Short-term safety profile of rhG-CSF in healthy donors

The administration of rhG-CSF to patients affected by hematologic diseases or cancer after chemotherapy or bone marrow transplantation is usually well tolerated and safe. Moreover, prolonged therapy with rhG-CSF, for months to years, given to patients with severe aplastic anemia or chronic neutropenia did not show any severe or cumulative toxicity.[6] In the last few years, the short-term safety profile of rhG-CSF in healthy donors has been investigated by several authors and the data so far accumulated reveal that such stimulation is well tolerated.[32] Anderlini *et al.* reviewed the clinical data related to peripheral blood stem cell collection in filgrastim-mobilized (6 μg/kg subcutaneously every 12 hours for 3-4 days) healthy donors who underwent leukapheresis for a target collection of $> 4\times10^6$/kg $CD34^+$ cells/kg recipient's body weight. The main adverse effects in 341 of 350 donors evaluable for toxicity of filgrastim were: bone pain in 84%, headache in 54%, fatigue in 31%, and nausea in 13%. These symptoms were rated as moderate to severe by 50% of the donors and resolved within 2-3 days after discontinuation of filgrastim. Analgesics, mainly acetaminophen, were administered to 71% of the donors. Less than 5% of the donors experienced non-cardiac chest pain, local reaction at the injection site, insomnia, dizziness or low-grade fever. Only 2 donors (0.5%) were withdrawn from donation because of severe toxicity: one because of headache/nausea and the other because of generalized bone pain.[18] In a retro-

spective comparison of 2 mobilizing doses of rhG-CSF for allogeneic PBSC (2×5 μg /kg vs 2×8 μg /kg for 5 days), grade III bone pain, headache and fatigue occurred only in the group receiving the 2×8 μg/kg dosage regimen.[33]

The expansion of the total number of myeloid cells is associated with a reversible and asymptomatic alteration of some biochemical parameters such as increases of serum levels of alkaline phosphatase (ALP), lactate dehydrogenase (LDH), uric acid and decreases of serum potassium, magnesium and cholesterol.

Overall, discontinuation of leukapheresis from a healthy donor because of severe toxicity is a rare event (1-3%) but strict monitoring and precise eligibility criteria are needed to avoid the underestimation of potential risks of stimulation with rhG-CSF. Platzbecker *et al.* found a 1.1-fold increase of splenic length and width during mobilization of 91 healthy donors (unrelated, 84; related, 7) with 7 μg/kg day × 5 days of lenograstim; this is equivalent to a 30%-increase of splenic volume over that before rhG-CSF stimulation.[34] Although no correlation was observed between changes of splenic diameters and WBC, neutrophil or platelet count; number of $CD34^+$/μL; concentration of LDH, ALP, AST, ALT; donor age and no case of splenic rupture occurred, it is conceivable to recommend a dose reduction of rhG-CSF in case of an excessive increase of WBC defined as more than 70×10^9/L, advise against extreme physical activity during rhG-CSF stimulation and avoid such stimulation in cases of recent infection, and chronic or recurrent inflammatory or thrombotic diseases. The 2 cases of splenic rupture so far reported were observed in one donor stimulated with 16 μg/kg/day of rhG-CSF over 6 days who reached a peak WBC count of 75×10^9/L and in another donor with concomitant Epstein-Barr virus infection.[35,36] Other single reports have underlined the risk of exacerbation of inflammatory diseases or induction of a hypercoagulable state.[32] Moreover, studies on primates showed that consecutive WBC counts above 100×10^9/L are associated with cerebrovascular hemorrhage.[20]

Side effects of PBSC apheresis

Peripheral blood $CD34^+$ cells are collected by large volume leukapheresis processing 3-6 times the donor's total blood volume in order to obtain at least 4×10^6 $CD34^+$ cells per recipient's body weight. Higher doses of $CD34^+$ cells (>8.0×10^6/kg) have recently been correlated with faster hematopoietic engraftment but at the risk of a higher incidence of chronic GvHD and without any advantage on relapse rate and overall survival.[37] Usually, one leukapheresis allows collection of the target $CD34^+$ cell dose in nearly 70% of cases.[18,21] Hypocalcemia may develop during large volume leukapheresis due to anticoagulation with acid-citrate-dextrose-A and the donor may require supplementation with calcium chloride. A slight deficiency of magnesium or potassium is also possible. Inadequacy of the peripheral venous access in permitting a consistent blood flow rate during apheresis is an important limiting factor in pediatric donations. On this subject, a recent report from the National Bone Marrow Program showed that a central venous line was needed in 41 of 395 (10%) G-CSF-stimulated unrelated PBSC donors. The venous access site was: femoral in 16 cases, forearm in 14 cases and inguinal in 11 cases. Most of the cases (37/41, 90%) involved female donors.[19]

Post-donation cytopenia is a common transient adverse effect observed in PBSC donors, partly related to the large volume leukapheresis, which removes large numbers of *mobilized* progenitor cells. In a multicenter prospective trial by Confer *et al*.[19] that evaluated feasibility and safety of unrelated PBSC in 395 donors, a median reduction of platelet count of 84×10^9/L below the baseline value was observed with each apheresis. Overall, 100 of 288 (35%) unrelated PBSC donors investigated had a platelet count < 100×10^9/L after day-6 leukapheresis but no bleeding occurred. At 1 month after donation the reductions below baseline value of WBC, hemoglobin and platelets were 0.7×10^9/L, 0.5 g/L and 12.3×10^9/L, respectively. A complete recovery to pre-donation baseline blood counts was found in all 111 donors who were followed-up at 1 year. These hematologic changes have no impact on the donor's mobilization efficiency and it has been demonstrated that a second PBSC collection is feasible with a collection yield similar to that of the first donation.[38,39]

Long-term safety issues

One of the major concerns regarding the use of rhG-CSF in unrelated healthy donors is the uncertainty about its possible role in triggering malignancy, in particular myelodysplastic syndrome and acute myeloid leukemia. The question has been raised by the experience in patients with chronic neutropenia and severe aplastic anemia but the evidence is weak because these diseases show *per se* a predisposition to develop clonal abnormalities regardless of any rhG-CSF treatment.[32,40,41] Moreover, in a recent retrospective study of 144 patients with aplastic anemia, Locasciulli *et al.* found that

the addition of large doses of G-CSF (36,000 μg/patient) over a 6-month period to cyclosporine and antilymphocyte serum did not increase the actuarial risk of cytogenetic abnormalities or malignancy after a median follow-up of 46 months in the G-CSF group (range 6.6-132.8).[42] Two retrospective studies have been performed so far on this subject, both in related allogeneic PBSC donors. Cavallaro *et al.* contacted 95 of 101 rhG-CSF-stimulated PBSC donors after a median follow-up of 43 months (range 35-57). No case of leukemia or myelodysplasia was encountered while the resulting blood counts in 70 of 95 donors were normal at a median follow-up of 40 months (range 16-70).[43] Likewise, no cases of leukemia or myelodysplasia were reported by Anderlini *et al.* in 281 donors after a median follow-up of 39 months (range 7-80).[44] Although the extension of such data to healthy unrelated donors carries the risk of not highlighting the role of genetic predisposition to malignancy among siblings and family members, the data so far accumulated argue against any risk from 4-5 days of exposure to G-CSF in developing leukemia. Currently, caution in more definitive conclusions is still warranted and studies on larger cohorts of donors for longer periods of time (10 years or more) are needed before any doubts can be put completely to rest.

Conclusions

Donor stimulation with rhG-CSF for $CD34^+$ cell mobilization into peripheral blood is increasingly being used in allogeneic hematopoietic stem cell transplantation. The rhG-CSF stimulation is generally well tolerated and the donor PBSC have the potential to replace bone marrow as the preferred source of hematopoietic cells for transplantation, as either related or unrelated grafts. The more significant or potential advantages are avoidance of general anesthesia and hospitalization for the donor and a stronger graft-versus-leukemia effect for the patient. The data so far accumulated show that 3-5 days of stimulation with G-CSF allows collection of an adequate cell-dose for transplantation in nearly 2/3 of procedures. Transient cytopenia (in particular, thrombocytopenia) is frequent but usually asymptomatic and self-limited. The donor's safety must be preserved by applying the eligibility criteria for blood donation, excluding potential candidates with inflammatory, autoimmune, and thrombotic/atherosclerotic underlying diseases and/or a family history of MDS, AML or Hodgkin's lymphoma. The long-term safety of G-CSF still remains an unresolved question that could be classified in the framework of international cooperation between the major national and international registries. Moreover, more effort is needed to assess the immunologic differences between PBSC and BM allografts, particularly in the unrelated setting.

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Role of a hematopoietic stem cell transplant registry in childhood: the experience of the Associazione Italiana Ematologia Oncologia Pediatrica (AIEOP)

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Hematopoietic stem cell transplantation (HSCT) is an established successful therapy for a great number of both congenital and acquired disorders of the lymphoid and hematopoietic system, as well as for solid tumors, in children. Information organized in observational databases and derived from large series of consecutive patients treated in several centers provides the resource for evaluating results of HSCT and offers a complementary approach for addressing different issues in this field. In fact, even though randomized, controlled clinical trials are the best way to evaluate efficacy of a certain therapy, they are difficult to perform due to the appropriate number of unselected patients who must be accrued to achieve reliable and precise estimates of outcome.[1] Moreover their realization implies significant cost and logistical difficulties. These facts combined with availability of new technologies for handling information provided the launching pad to explore alternative methods of investigation, such as retrospective analyses performed through information collected and stored in observational databases. Through such registries, besides deriving data on the efficacy of HSCT in different disorders, it is also possible to retrieve relevant information for designing phase III confirmatory trials and for calculating estimates of outcome necessary to define the sample size and the study plan. Moreover, periodic and systematic assessments of overall results in specific disease are informative and useful for physicians, regional and/or central governmental agencies, and other individuals or organizations involved in health-care.

Since transplantation of hematopoietic progenitors in our country is rapidly evolving, knowledge of variation over time is essential for correct interpretation of current practice of HSCT in Italy. Awareness of results obtained with HSCT on a national scale also provides an updated basis for decision-making in health-care planning and management. This report offers relevant information on transplant activity for pediatric patients in Italy.

Design and Methods

Since 1985, the Italian Bone Marrow Transplant Registry of the Associazione Italiana Ematologia Oncologia Pediatrica (AIEOP BMT Registry) has collected and stored data from centers performing HSCT in pediatric patients nationwide. Currently, 23 centers report data to the AIEOP BMT Registry. About a half of teams (47%) perform both allogeneic and autologous transplants. The percentage of Centers fulfilling the GITMO/EBMT criteria to be accredited for either autologous or allogeneic transplant is 68% and 63%, respectively.

Participating teams are required to register all consecutive transplants adopting a web-based database created by Oracle and protected by IANUS® technology, implemented at the *Italian Interuniversity Computing Center* (CINECA) in Bologna, Italy. Data are stored in a central database (AIEOP BMT Registry), organized at the AIEOP Operation Office, which is structurally integrated with other specific, disease-oriented national databases. Second transplants for disease relapse and planned double procedures for the same patient are counted, whereas second transplants for graft failure are not.

Using these data, linked with information from other disease-oriented databases, the Statistical Center, at the AIEOP Operation Office, periodically

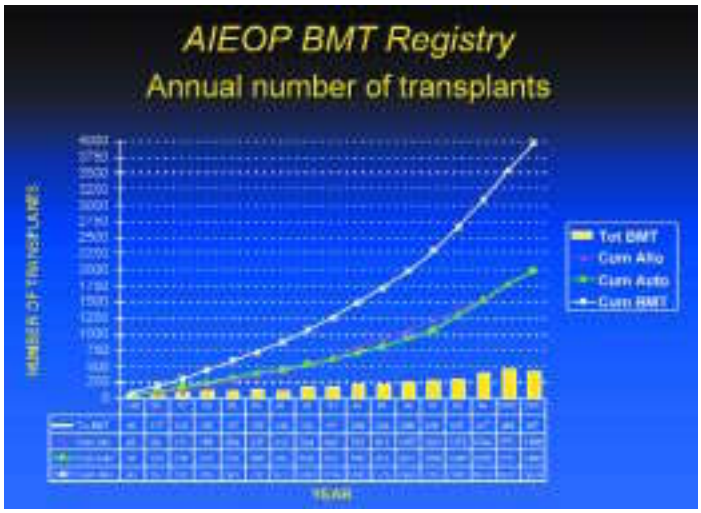

Figure 1. Annual number of transplants. Abbreviations: Tot BMT, total number of transplants per year; Cum Allo, cumulative number of allogeneic transplants; Cum Auto, cumulative number of autologous transplants; Cum BMT, cumulative number of transplants.

prepares and distributes reports summarizing current use and outcome of allogeneic and autologous HSCT. Moreover, these data have provided the basis for several retrospective analyses[2-4,10-13] and prospective trials in the last 5 years)[5-9] published by the Italian AIEOP BMT group.

Results and comments

Transplant figures

Use of blood and marrow transplants continues to increase constantly. From January 1985 to December 2001, a total of 3,979 bone marrow (BM), peripheral blood (PB) or umbilical cord blood (CB) transplants performed on 3,436 patients were reported: 1,989 (50%) were allogeneic transplants and 1,990 (50%) were autologous. In the last 3 years, the number of allogeneic and autologous transplants per year has been approximately the same (Figure 1). The AIEOP BMT Registry database includes information for 21% and 10% of allogeneic and autologous transplants, respectively, performed per year in Italy. The monitored population represents more than 90% of procedures performed in children aged 0-17 years.

Stem cell source

Among allogeneic transplants, 1,755 (88%) were performed using BM progenitor cells, whereas the stem cell source was CB in 99 (5%), PB in 117 (6%), BM plus PB in 12, and BM plus CB allografts in 5. While the number of autologous procedures appears to have been increasing since 1997, there is a constant increment for allogeneic transplants since 1999 (Figure 1). The continuously increasing number of autologous transplants is likely due to several factors, including new disease indications and repeated procedures for a single patient. The increment in allogeneic transplants mainly reflects a greater availability of suitable unrelated donors together with an expansion of the indications for such transplants in specific diseases. Most patients given allogeneic transplants received bone marrow progenitor cells. There has, however, been a steady increase in the number of allogeneic transplants using hematopoietic cells collected from either peripheral or umbilical cord blood. Out of the autologous transplants, 861 (45%) were performed using BM, and 1,039 (35%) using PB hematopoietic stem cells alone or combined with BM. Currently, more than 80% of autotransplants use only hematopoietic progenitor cells collected from blood. In particular, in last 6 years, the use of PB as the source of stem cells for autografts has increased from 49% to 85%.

Main indications for HSCT

Figure 2 illustrates indications for childhood allogeneic and autologous HSCT in Italy. The most common indications for allogeneic and autologous transplants differ. Allogeneic transplants were performed in 1,316 (66%) patients with malignancy and in 673 (34%) children with non-malignant disorders. Acute leukemias, myelodysplasia and non-malignant diseases (aplastic anemia, immune deficiencies, inherited metabolic disorders) have been predominantly treated with allogeneic transplants. Indications for autologous HSCT were solid tumor in 1,266 (64%) cases, myelo-lymphoproliferative disorders in 702 (35%) cases (249 acute lymphoblastic leukemia, 317 acute myeloblastic leukemia, 129 lymphomas, 7 other leukemias), and non-malignant disease in 22 (1%) cases. Auto-

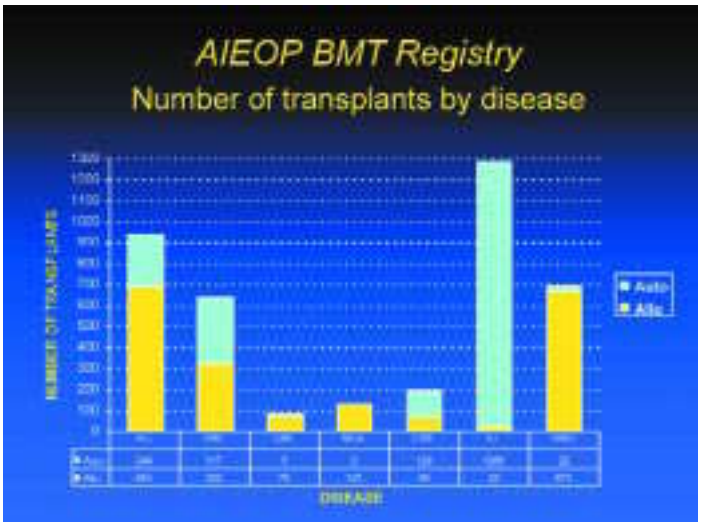

Figure 2. Number of transplants by disease. Abbreviations: Allo, allogeneic; Auto, autologous; ALL, acute lymphoblastic leukemia; AML, acute myeloblastic leukemia; CML, chronic myelogenous leukemia; MDS, myelodisplastic syndrome; LYM, lymphomas; ST, solid tumors; NMD, non malignant diseases.

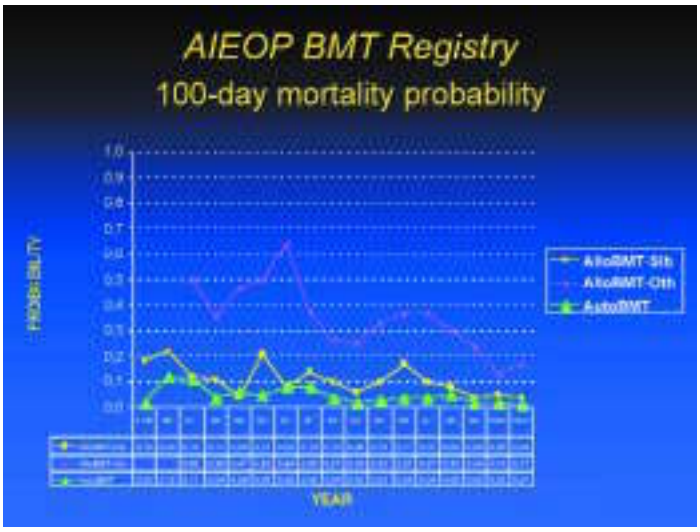

Figure 3. 100-day mortality probability. Abbreviations: AutoBMT, autologous; AlloBMT-Sib, allogeneic-sibling family donor, AlloBMT-Oth, allogeneic-other donors.

transplants are generally used for neuroblastoma, soft tissue sarcomas, and other malignancies, as well as for the treatment of patients with Hodgkin's and non-Hodgkin's lymphomas.

Donor type

Allogeneic procedures were performed using HLA-identical sibling donors in 1,211 cases (61%) and alternative donors (i.e. partially-matched relatives or unrelated donors) in the remaining 778 (39%) cases. Increasing availability of HLA-typed volunteer donors through world wide registries, as well as the establishment of cord blood banks has enabled increasing use of unrelated donors for stem cell transplants. At present, transplants from unrelated donors account for approximately 25% of all allogeneic transplants, and over 40% if only the last five years are considered.

Outcome

Transplant-related toxicity, measured by 100-day mortality, is reported in Figure 3. Allogeneic transplants are associated with risks of graft-versus-host disease (GvHD), infections and liver toxicity, resulting in higher early mortality. Over the period analyzed, among HLA-identical sibling transplants the 100-day mortality decreased from about 20% to 4%, whereas this value for allogeneic transplants from unrelated or partially-matched relatives donors decreased from more than 50% to less than 20%. Early mortality is lower following autotransplants, with values in the order of 1-2% in the last years.

Most children with acute lymphoblastic leukemia (ALL) are cured with conventional chemotherapy. Consequently, bone marrow transplant is general-

ly reserved for patients failing to benefit from conventional therapy, such as cases of relapse or second or subsequent remission, or patients in first remission with prognostic factors predicting a very high risk of failure with conventional therapy. Among 297 recipients of HLA-identical sibling transplants between 1985 and 2001, the 10-year% probabilities of event-free survival (95% CI) were 69.3% (59-79) for 97 transplants done in first complete remission (CR), 54.8% (47-62) for 200 transplants carried out in second remission, and 35.8% (23-49) for 54 transplants in a subsequent remission. Although associated with higher transplant-related mortality, unrelated donor transplants may be considered for patients with ALL who are unlikely to be cured by chemotherapy. Among 80 recipients of matched unrelated donor transplants for ALL in second remission 5-year probability of event-free survival (95% CI) was 38.1% (26-50).

Among recipients of HLA-identical sibling transplants for acute myeloblastic leukemia (AML) performed between 1985 and 2001, the 10-year probability of event free survival (95% CI) was 62% (51-73) for 141 transplants in first CR. Among patients receiving autologous transplants for AML between 1985 and 2001, the 10-year % probability of event free survival (95% CI) was 51.0% (44-58) for 237 patients in first CR at the time of transplant, and 38.2% (26-51) for 59 patients in second CR at the time of transplant.

This analysis also provides information on patients' outcome for each different diagnosis and stage of disease separately for each type of transplant. The data show that the vast majority of children given an allogeneic transplant for a non-malignant disease have been cured. Access to information on the five-year survival probability obtained from data on a large number of patients treated with either *routine* or clinical research protocols is valuable for counseling patients.

Conclusions

The present report provides information on number of transplants, donor source, donor type, disease type and outcome for childhood HSCT in Italy.

The two peculiar features of the AIEOP BMT Registry are that it is an exclusively pediatric database and that it has adopted shared technologies for information handling. Its structure, designed to organize information in a dedicated observational database, specific for pediatric HSCT, provides an important complementary approach to addressing issues in several fields of pediatric hematology and oncology.

In conclusion, an exhaustive, reliable and regularly updated database represents a useful tool for gathering information on the role of HSCT in children and for providing unbiased counseling of patients. Knowledge of current activity and ongoing trends on a national basis offers a platform for more effective health-care planning, better definition of transplant indications and improvement of the quality of medical research.

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Allogeneic bone marrow transplantation versus chemotherapy in childhood very high risk acute lymphoblastic leukemia in first complete remission: a controversial issue

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Background and Objectives. Improvements in the management of childhood acute lymphoblastic leukemia (ALL) did not prevent 20% to 30% of patients suffering from relapse. Moreover, the probability of relapse can rise up to 50% for some children presenting with very high risk (VHR) factors. Intensive chemotherapy and especially hematopoietic stem cell transplantation improved their outcome. The aim of this review is to assess the role of different approaches in the treatment of childhood VHR ALL on the basis of current data.

Evidence and Information Sources. Information on the ongoing international studies was obtained via Medline®. Preliminary data from a prospective cooperative study are mentioned.

State of Art. During the last decade, different definitions of VHR factors in childhood ALL have been a crucial issue, so that therapeutic results of single or multicenter studies were difficult to compare. All investigators agreed in adopting most aggressive treatments in patients with poor prognostic factors such as molecular biological markers, chromosomal abnormalities and biological factors including poor prednisone response and resistance to initial chemotherapy. Allogeneic bone marrow transplantation (AlloBMT) in childhood VHR ALL in first complete remission is expected to yield better event-free survival than chemotherapy. The lack of valid information in the current literature about the real impact of both chemotherapy and hematopoietic stem cell transplantation is essentially the result of the difficulty in setting up multicenter prospective studies. On the other hand, the principal biases of retrospective studies are the lack of homogeneous eligibility criteria, different first-line therapies adopted before AlloBMT and above all the *waiting time to transplant* which could have accounted for some of the survival advantage shown by AlloBMT patients compared to those treated with chemotherapy.

Perspectives. Preliminary results of an ongoing international prospective study are presented and compare favorably with previous reports. The current scenario serves as an example of how to reach a consensus in the controversial treatment of childhood VHR ALL.

Key words: allogeneic BMT, childhood, acute lymphoblastic leukemia.

In the 1990s data emerging from single or multicenter trials regarding the outcome of children with very high risk (VHR) acute lymphoblastic leukemia (ALL) in first complete remission (CR) showed event-free survival (EFS) ranging from 50 to 70% for patients treated with allogeneic bone marrow transplantation (AlloBMT)[1-6] and from 10 to 40% in those managed with chemotherapy.[6-11] Data varied according to different patient selection and different intensities of first-line protocols. Moreover the possibility of performing hematopoietic stem cell transplants from alternative donors made it more important to evaluate the real impact of each strategy in this cohort of patients.

Definition of VHR factors in childhood ALL

The definition of VHR criteria varied over the years and among international co-operative groups (POG, CCG, BFM, AIEOP, EORTC) which adopted different clinical, biological, and cytogenetic criteria [e.g. age, white blood cell (WBC)] count, T-immunophenotype combined with *poor* prednisone response, MLL rearrangements, BCR/ABL fusion, induction failure and, more recently, minimal residual disease persistence).[12-19] Moreover different combinations of risk factors were used to stratify patients for first-line protocols in order to overcome possible leukemic resistant clones; this strat-

egy was reflected by variations in EFS obtained by different Institutions.[10,15,19-21]

Value of a transplantation strategy for VHR childhood ALL in first CR

Allogeneic hematopoietic stem cell transplantation from a sibling donor in the treatment of VHR ALL in first CR is generally accepted; the role of transplantation from alternative donors is still to be assessed. Few studies in the past compared AlloBMT and chemotherapy. Single center studies, such as those of Brochstein[1] and Sanders,[2] and a multicenter trial reported by Bordigoni,[3] showed promising results for AlloBMT patients. However, Chessels on behalf of the MRC in 1992,[4] did not find a statistical significant difference in EFS between AlloBMT patients and chemotherapy-treated patients for high-risk childhood ALL in first CR. In 1996 Saarinen[6] demonstrated, in a case-control study of 22 AlloBMT recipients versus 44 matched controls, a superior outcome for patients undergoing the AlloBMT procedure (10 year DFS of 73% vs. 50%); however, eligibility criteria for this study selected a group of patients without intermediate high-risk features at diagnosis.

An Italian co-operative study[5] based on homogeneous VHR criteria by means of a matched design to control for both known VHR prognostic factors and time to-transplant bias, showed no significant statistical difference between the two groups (4 year DFS of 59% and 48% for AlloBMT and chemotherapy-treated patients, respectively). Despite this, the AlloBMT curve remained stable reaching a plateau after one year whereas chemotherapy curve was more likely to fall, since patients continued to experience relapse even after 4 years. Finally, some few promising data concerning alternative donor transplantation (matched or partially mismatched unrelated donor, haplotype mismatched related donor, cord blood transplantation)[22-27] in childhood ALL in first CR have recently been reported but need confirmation in larger series of patients prospectively studied before any conclusions are drawn from them.

Recent international prospective studies for VHR childhood ALL in first remission

Efforts were recently made by the I-BFM-SG to better define the impact of VHR factors in order to tailor risk-adapted first-line protocols, including AlloBMT. A subset of VHR patients (roughly 15% of the total ALL patients) were retrospectively identified on the basis of common criteria, including clonal translocations as t(9;22) or t(4;11), T-immunophenotype associated with hyperleukocytosis and poor response to prednisone. Despite very intensive first-line protocols, comprising high-dose chemotherapy blocks of non-cross-resistant drugs, neither the BFM-ALL-90[10] nor the AIEOP-ALL-91 study[9] had achieved an EFS higher than 40% for VHR ALL children. Once again one should emphasize that only a homogeneous selection of patients would allows an unbiased comparison between chemotherapy and AlloBMT.

In 1995 the EBMT and I-BFM-SG set up a prospective randomized trial with the aim of comparing the role of AlloBMT and chemotherapy in the treatment of VHR childhood ALL in first CR. The homogeneous criteria adopted in defining VHR patients at diagnosis were as follows: 1) cytogenetic abnormalities such as t(9;22) or t(4;11); 2) poor response to prednisone associated with either T-ALL or WBC higher than 100×10^9/L at presentation, and 3) induction failure. First-line protocols were similar between groups, patients and potential related donors were HLA typed at presentation, allowing a sort of biological randomization within the attainment of first CR. Patients with suitable HLA A, B, DR compatible related donors were assigned to the AlloBMT arm and were to be transplanted early after consolidation of first CR. Crucial issues in the statistical design of this co-operative study were the recruitment of a sufficient number of patients, the *intention-to-treat* analysis, and the adjustment for waiting time to transplant in the *performed treatment* analysis. The final analysis is ongoing and will be concluded before the end of 2002. Preliminary evaluation reported an extremely high participation in the study; out of the 392 VHR ALL patients recruited over 5 years, 336 were eligible/evaluable for the analysis, and a median follow-up of 50 months was reached. Seventy patients had a compatible related donor, while 266 did not. Actually, 244 patients received chemotherapy, 20 of them despite having a compatible related donor, amd 92 underwent transplantation, 50 of them from their compatible related donor and 42 from alternative donors. The cohort of patients with a suitable donor had a significant advantage versus the cohort of patients with no donor, with a 5 year DFS of 58 which compares favorably with historical data. In particular, the subset of patients with a poor response to prednisone and T-immunophenotype and/or hyperleukocytosis at diagnosis reported a 4-year DFS of 60%. The outcome of other subgroups of patients, such as those with genetic translocations associated with prednisone poor

response or those who failed to obtain first CR, remained dismal. Recently MRD persistence two to three months after diagnosis of childhood ALL seemed to identify a new negative prognostic factor which could further contribute to allocate patients into each therapeutic option.[28] On the basis of this and other reports, eligibility for transplantation from conventional or alternative donors should be considered in a more dynamic way, according to most recent reports showing updated chemotherapy outcomes.

Conclusions

In view of the most recent therapeutic results in VHR ALL children, it is mandatory to update expected outcome according to the continuous progress of both AlloBMT and chemotherapy reached by intergroup co-operative studies. Future challenges include the reduction of the relapse rate, constituting the most frequent cause of treatment failure in childhood VHR ALL. On the other hand, a reduction of transplant-related mortality by means of better supportive therapy would extend eligibility for new prospective clinical trials of transplantation from alternative donors.

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Unrelated donor marrow transplantation in childhood: a report from the Associazione Italiana Ematologia e Oncologia Pediatrica (AIEOP) and the Gruppo Italiano per il Trapianto Midollo Osseo (GITMO)

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Background and Objectives. Unrelated donor bone marrow transplant (UD-BMT) has become an attractive, alternative source of hematopoietic cells for patients lacking a matched sibling. The aim of this paper is to report on 520 patients below 19 years of age undergoing UD BMT in 31 Italian centers between September 1989 and December 2001, and to focus on the results achieved in the 423 patients grafted before December 2000.

Designs and Methods. In 1989 the Italian Bone Marrow Transplant Group (GITMO) and the Italian Association for Pediatric Hematology and Oncology (AIEOP) established the Italian Bone Marrow Donor Registry (IBMDR) to facilitate donor search and marrow procurement for patients lacking an HLA identical sibling. By the end of December 2001, 296,720 HLA-A, B typed volunteer donors had been cumulatively registered and 3,411 searches had been activated for Italian patients. At least one HLA-A, B, DRB1 matched donor was found for 54% of the patients and 520 UD BMTs were performed in patients below 19 years of age before December 2001. Since 1999 more than 90% of the patients ≤14 years old, and more than 50% of the patients 15-18 years old undergoing UD BMT have been treated in AIEOP institutions. In 50% of the cases donors were found in the IBMDR, and in 50% they were found in 14 other Registries. The average time from search activation to transplant was 6 months for diseases other than chronic myeloid leukemia (CML), while for CML it was 8.7 months.

Results. Actuarial 100-day transplant-related mortality (TRM) was 32% in patients grafted between 1989 and 1997, and 21% for patients grafted after 1998 (p=0.003). Twenty-eight per cent of the patients developed grade III or IV acute graft-versus-host disease (GvHD), and 20% developed extensive chronic GvHD. The rate of disease-free survival at three years was 37% for patients with acute lymphoblastic leukemia, 38% for acute myeloid leukemia or myelodysplastic syndrome patients, 59% for patients with inborn errors, and 51% for patients with CML.

Interpretation and Conclusions. We conclude that the IBMDR has benefited a substantial number of patients lacking a matched sibling and has facilitated the recruitment of UDs into the international donor pool. Results show a positive trend after 1998, mainly due to a decrease in transplant-related-mortality.

Key words: unrelated donor BMT, childhood leukemia, inborn errors.

Allogeneic bone marrow transplantation (BMT) from HLA-identical siblings has become an effective treatment for patients with hematologic malignancies, syndromes of marrow failure, and various inborn errors.[1-3] Since only one out of three patients has a suitable matched sibling, several investigators have explored the use of alternative sources of hematopoietic stem cells, including

HLA-mismatched family members,[4] unrelated donors (UD),[5] or cord blood (UCBs) units.[6] The *Italian Bone Marrow Transplant Group* (GITMO) and the *Italian Association for Pediatric Hematology and Oncology* (AIEOP) established the *Italian Bone Marrow Donor Registry* (IBMDR) in 1989 to facilitate donor search and marrow procurement for patients lacking an HLA identical sibling. The data regarding the first 10 years of UD BMTs performed in Italy were reported in 2001.[7] Three hundred and twenty-nine out of 696 patients were less than 19 years old. The aim of this paper is to report on 520 patients below 19 years old given an UD BMT in 31 Italian centers between September 1989 and December 2001, and to focus on the results achieved in the 423 patients grafted before December 2000.

Design and Methods

IBMDR

The IBMDR was established as a collaborative effort among the Regional Tissue Typing Laboratories. It includes 17 local Registries and 100 donor centers for the HLA class I and class II typing of volunteer donors. Its principles and policies have been previously described.[5,7]

Donor recruitment

Over the last 10 years more than 200,000 new donors have been added to the Registry. Currently, the IBMDR file contains data concerning approximately 296,720 volunteers. All of them are HLA-A, B serologically typed, while HLA-DR serological typing has also been performed on 119,000 (43%) of these registered donors. The DRB1 typing by PCR technique of 23,100 (7.8%) donors is also available. Once a month the IBMDR electronically sends its donor HLA phenotype file to the *Bone Marrow Donors World Wide* (BMDWW) directory, which included more than 7,500,000 donors in its December 2001 edition.[8] All the Italian transplant centers involved in this program are accredited through the GITMO to the IBMDR, to European Registries, and to the *National Marrow Program of the United States of America* (NMDP), according to the *World Marrow Donors Association* standards.

Matching criteria

Before December 1991, most transplant centers based their matching criteria on HLA-A, B, DR identity using serologic testing. After January 1992, class II antigen matching was confirmed by DNA techniques as previously described.[5,7] Since 1998, most centers extensively study class I and class II alleles by DNA techniques. Strategies to select the best donor are decided by the individual institutions.

Data collection and statistical analysis

Essential data regarding all donor-recipient pairs, and information concerning the harvesting procedure were obtained by the IBMDR data center. Information on the BMT procedure and on the follow-up of the recipients are collected every year by the AIEOP-BMT Registry for patients treated in Pediatric institutions and from the GITMO Registry for patients treated in adult institutions. Since 1997, the AIEOP-BMT Registry has also transferred data on the activity of each center to the GITMO Registry, and through it to the European Blood and Marrow Transplantation (EBMT) Registry.[9]

Life-table analysis was conducted according to the Kaplan and Meier method.[10] Disease-free survival (DFS), and 100-day transplant-related-mortality (TRM) were assessed. In the evaluation of DFS, recurrence and death due to any cause, whichever came first, were counted as failure. In calculating TRM, death due to any transplant-related cause occurring in the first 100 days after BMT was considered a failure. For the purpose of these analyses, patients were censored at the date of last follow-up, if no failure was reported. Data were updated as of December 31st, 2001.

Results

The search

Between September 1st 1988 and December 31st 2001, the search for an UD was started for 3,411 patients. Half of them were activated during the last three years. During the last 12 months, a formal search was started each month for approximately 40 new Italian patients.

Probability of finding a matched donor

At least one HLA-A, B, DRB1 matched donor was found for 54% of the patients. At least one antigen mismatched donor was found for 66% of the patients. At least one HLA-A, B, CW, DRB, DQA, DQB matched donor was found for 28% of the patients. As of December 2001, 520 Italian patients below 19 years of age had undergone UD BMT in one of the 31 transplant centers participating in this program. Half of them were transplanted during the last 3 years. In 2001, each month approximately 8 new patients underwent UD BMT. Details on the activity of the 31 transplant centers are reported in the *Appendix*. The characteristics of donors and recipients listed according to the type of disease that led to transplant are reported in

Table 1. Characteristics of donors and recipients according to the type of disease that led to transplant.

Disease	No. pts.	Age (years) donor Median	Age (years) donor Range	Age (years) Recipient Median	Age (years) Recipient Range	Gender Donor M	Gender Donor F	Gender Recipient M	Gender Recipient F
Neoplastic	310								
Acute Lymphoblastic Leukemia	163	34	20-57	10	1-18.88	91	72	107	56
1st remission	28								
2nd remission	96								
≥3rd remission	39								
Acute Myeloid Leukemia	46	33	22-55	9.03	1.42-18.66	30	16	30	16
1st remission	18								
2nd remission	19								
≥3rd remission	9								
Myelodysplasia	50	38	22-55	7.60	1.21-18.58	27	23	23	27
Chronic Myeloid Leukemia	47	35	24-58	14	6-18.84	24	23	29	18
1st chronic phase	33								
2nd chronic phase	5								
Accelerated phase	7								
Blast crisis	2								
Non-Hodgkin's lymphoma	4	35	32-38	13.6	9-16	4		4	
Non neoplastic	113								
Severe aplasia	10	39	24-51	10.65	3.79-15.74	5	5	3	7
Inborn errors	103	35	20-52	4.05	0.13-18.36	56	47	68	35
Total	423	35	20-58	9.02	0.13-18.88	237	186	264	159

Table 1. Four hundred and eleven out of 520 patients underwent UD BMT in an exclusively pediatric AIEOP center, while 109 were treated in adult institutions.

After 1999, 191/214 (89%) patients 14 years old or less, and 21 /55 (38%) patients from 15 to 18 years old underwent UD BMT in an exclusively pediatric AIEOP center. The distribution of patients <14 and >15 years old is shown in the *Appendix*.

The number of patients grafted per year for each disease increased progressively, while a decrease in the number of procedures for chronic myeloid leukemia (CML) was observed during the last two years.

Donor registries

In Italy, the first UD BMT performed on a patient less than 19 years old was made possible in March 1989 by a donor listed with the *Anthony Nolan Research Foundation*. The first UD marrow provided by the IBMDR was grafted in February 1991. By December 2000, 213 donors had been provided by the IBMDR and 210 by other Registries, as shown in Table 2.

Donor recipient matching

Eighty per cent of the recipients were HLA-A, B, DRB1 matched, while 17% were mismatched for one antigen. Details on donor-recipient matching are reported in Table 3.

Table 2. Registries and the number of donors they provided to Italian patients.

IBMDR	213
NMDP USA	57
German	54
A. Nolan	45
French	24
Canadian	7
Dutch	6
Swiss	5
Australian	3
Belgian	3
Swedish	2
Austrian	1
Finnish	1
Norwegian	1
Spanish	1
Total	423

IBMDR, Italian Bone Marrow Donor Registry; NMPD USA, National Marrow Donor Program United States of America.

Preparative regimen and graft-versus-host disease prophylaxis

The preparative regimen and GvHD prophylaxis varied depending on the underlying disease, on the transplant center, and over time. Details are reported in Table 4. Briefly, the preparative regimen of 230 out of 421 patients whose data were available, included radiation which was either preceded or followed by one or more drugs. One hundred

Table 3. Matching.

Matching	*No. of pairs*	*%*
A,B,DRB1 =	339	80
1 A locus antigen ≠	29	7
1 B locus antigen ≠	21	5
1 DRB1 antigen ≠	21	5
> 1 antigen ≠	5	1
Unknown	8	2
Total	423	100

Table 4. Conditioning regimens.

	No. of pts.
Neoplastic diseases	310
Radiation-containing regimens	210
Non-radiation-containing regimens	98
Unknown	2
Non-neoplastic diseases	113
Radiation-containing regimens	20
Non-radiation-containing regimens	93
Total	423

and ninety-one patients were treated with a non-radiation-containing regimen. Two hundred and eighty-three patients received *serotherapy* (ATG or Campath 1G)-containing GvHD prophylaxis, including cyclosporin (CSA) and *short* methotrexate, while 62 other patients received non-serotherapy-containing GvHD prophylaxis. Forty patients received other GvHD prophylaxis. Data concerning the remaining 38 patients were not available.

GvHD

Three hundred and five of 374 evaluable patients (81.5%) developed grade I (98, 26.2%), grade II (100, 26.7%), grade III (63, 16.8%), or grade IV (44, 11.7%) GvHD. Chronic GvHD developed in 106/269 (39.4%) evaluable patients, and 53 of them (19.7%) had extensive disease.

Transplant-related mortality (TRM)

Altogether 151 patients died of TRM. Between 1989 and 1997, 57 out of 196 patients died within the first 100 days after BMT, and 34 died more than 100 days after BMT. Actuarial 100-day TRM was 32% (CI 26-82). Between January 1998 and December 2000, 42 out of 229 patients died within the first 100 days after BMT, and 18 patients died more than 100 days after BMT. Actuarial 100-day TRM was 21% (CI 15-27) (p=0.003). Timing and causes of TRM are reported in Table 5.

Acute lymphoblastic leukemia (ALL)

One hundred and sixty-three patients with ALL underwent UD BMT. The median interval between search activation and transplant was 5.4 months (range 1.3-19.2). Altogether 60/163 patients died of TRM. Actuarial 100-day TRM was 29% (CI 23-37). Leukemia relapse occurred in 43 patients at a median of 4.8 months (range 10 days-17.3 months) after BMT. Three-year DFS from time of transplant was 37% (CI 29-44). DFS probability of patients who underwent UD BMT before or after 1998 was respectively, 28% (CI 19-39) and 47% (CI 35-57) (p=0.0008).

Acute myeloblastic leukemia (AML) and myelodysplasia (MDS)

Forty-seven patients with AML, and 50 with MDS underwent UD BMT. The median interval between search activation and transplant was 5.1 months (range 0.3-44.1). Altogether 38 patients died of TRM. Actuarial 100-day TRM was 32% (CI 23-43). Disease progression occurred in 26 patients at a median of 3.7 months after BMT (range 2 days-37.5 months). Three-year DFS was 38% (CI 28-47). It was 27% (CI 14-42) and 46% (CI 32-58) for patients undergoing UD BMT before and after 1998, respectively (p=ns).

Chronic myeloid leukemia

Forty-seven patients with CML underwent UD-BMT. The median interval between search activation and transplant was 8.7 months (range 2.5-months-3.4 years). Altogether 20 out of 47 patients died of TRM. Actuarial 100-day TRM was 22% (CI 12-37). CML recurrence occurred in 6 patients at a median of 9.35 months (range 1.1 months-4.8 years). Three-year DFS from time of transplant was 51% (CI 36-54).

Non-malignant disorders

Before 1998 only a few patients with severe aplastic anemia, mostly in advanced phases, underwent UD BMT, and the results were disappointing. However, preliminary data of a prospective multicenter trial designed for patients in earlier phases seem encouraging. One hundred and three patients with inborn errors underwent UD BMT. The underlying diagnoses are reported in Table 6. The median interval between search activation and transplant for these patients was 6.2 months (range 0.6 months-9.6 years). Altogether 30 patients died of TRM. The 100-day TRM rate was 15% (CI 9-24). Graft failure and recurrence of the underlying disease occurred in 18 patients at a median of 1 month after BMT. Three-year DFS was 59% (CI 48-67).

Table 5. Timing and causes of transplant-related mortality before and after 1998.

	No. of patients			
Year of BMT	1989-1997 (n=196)		1998-2000 (n=227)	
Cause of death	First 100 d After BMT	After 100 d From BMT	First 100 d After BMT	After 100 d From BMT
GvHD	14	13	14	5
Interstitial pneumonia	15	7	9	5
Infections	6	4	5	3
Hemorrhage	4	2	3	2
Organ failure	3	3	2	1
ARDS	4	–	3	–
VOD	4	–	2	–
EBV LPD	2	1	2	–
Encephalopathy	1	2	–	–
Unknown	–	–	2	2
Graft failure	1	1	–	–
Other toxicity	1	–	–	–
2nd tumour	–	1	–	–
Cardiac failure	2	–	–	–
Total	57	34	42	18

BMT, bone marrow transplant; GvHD, graft-versus-host disease; ARDS, acute respiratory distress syndrome; VOD, veno-occlusive disease; EBV LPD, Epstein Barr lymphoproliferative disease.

Table 6. Diagnosis and follow-up of 103 patients with inborn errors undergoing UD BMT.

Diagnosis	*No. of pts.*	*ADF*	*AWD*	*DDF*	*DWD*
SCID	22	18	–	–	4
Thalassemia	22	13	5	4	–
Fanconi's Anemia	14	6	–	3	5
Storage disorders	12	9	–	1	2
HLH	9	4	–	3	2
Wiskott Aldrich S.	9	6	1	2	–
Osteopetrosis	4	2	1	–	1
Blackfan-Diamond Anemia	3	2	–	1	–
Shwachman Syndrome	1	1	–	–	–
Chediak Higashi Syndrome	1	1	–	–	–
Kostmann Syndrome	1	–	–	1	–
Glanzman Thromboasthenia	1	1	–	–	–
Granulomatous Chronic Disease	1	–	–	1	–
Porphyria	1	1	–	–	–
Di George Syndrome	1	–	–	1	–
Dyserythropoietic anemia	1	–	–	1	–
Total	103	64	7	18	14

ADF, alive disease-free; AWD, alive with disease; DDF, dead disease-free; DWD, dead with disease; SCID, severe immunodeficiency; HLH, hemolymphohistiocytosis.

Discussion

In this paper we report information on IBMDR activity, on patients less than 19 years of age undergoing UD BMT in Italy, on donor origin and on the results we obtained.

Surprisingly, UD BMTs performed on children in Italy account for 45% of all UD transplants. The increasing number of procedures performed over the years is related to the greater number of searches that have been activated through the IBMDR, and to the higher number of donors. This experience shows that a great deal of people are, indeed, willing to donate their bone marrow in Italy and around the world. This has been made possible thanks to co-operation between volunteers and medical and non-medical staff.

Our data show that a suitable donor was found for 66% of the patients. This is approximately 50% higher than the figure we reported in 1996.[5] The recruitment of more than 100,000 Italian donors over the last 5 years will probably further increase the number of patients who will find an UD in the IBMDR. In our study the average time from search activation to transplant was 5 to 6 months for diseases other than CML, while the median interval between search activation and transplant for CML was 8.7 months.

Actuarial 100-day TRM was 32% in patients grafted between 1979 and 1998, and 21% for patients grafted between January 1999 and December 2000 (p=0.003). The long time required for the search, and the salvage chemotherapy before transplant are likely the main reasons for the high TRM rate in patients with acute leukemia. Earlier transplant and decreased early TRM substantially changed the outcome of grafted patients after 1999. The reasons for this improvement are mostly correlated to refinements of HLA-typing techniques, to GvHD prophylaxis, and to improved supportive therapy. Our data show that, given these improved results, the number of patients offered an UD BMT increased during the years for all indications other than CML. The high risk of TRM, as well the chance of severe GvHD were causes of concern for UD BMT early in the course of the disease. Moreover, recently, several patients have been enrolled in prospective studies aimed at assessing the efficacy of interferon-α or STI. It must be noted that the decreased risk of TRM observed in patients grafted after 1999 has produced a significant improvement in the prognosis of patients receiving BMT from an *extensively* matched UD after a TBI-containing regimen.[15] This is why the search for an UD for CML patients less than 19 years old should be activated at diagnosis. An initial trial of STI could be appropriate for all patients.

Appendix. Transplant Centers. Number of unrelated donor transplants by year in patients less than 19 years of age between March 1989 and December 2001, and the main investigators.

	Period	No. of BMTs					
		≤1998		≥1999			Main
	Age at BMT (yrs)	0-14	15-18	0-14	15-18	Total	investigators
AIEOP Institutions	CIC Transplant Center						
*	Pavia, Policlinico S. Matteo – Clinica Pediatrica	57	6	57	5	125	F. Locatelli G. Giorgiani
*	Genova, Istituto G.Gaslini – Dipartimento Ematologia e Oncologia Pediatrica	33	5	34	6	78	G. Dini E. Lanino
*	Brescia, Clinica Pediatrica	28	–	23	–	51	F. Porta E. Mazzolari
*	Padova, Clinica Pediatrica	14	3	25	2	44	C. Messina S. Cesaro
*	Monza, Ospedale S. Gerardo, Clinica Pediatrica	19	2	17	3	41	C. Uderzo A. Balduzzi
*	Bologna, Clinica Pediatrica	5	1	22	1	29	A. Pession A. Prete
*	Torino, Clinica Pediatrica	16	4	5	3	28	F. Fagioli E. Vassallo
⊗	Cagliari, Serv. Ematologia Osp. R. Binaghi	8	4	4	5	21	G. La Nasa R. Floris
⊗	Roma, Univ. La Sapienza Catt. Ematologia	8	1	7	2	18	W. Arcese A. Iori
°	Pesaro, Divisione Ematologia	5	–	3	1	9	C. Giardini
⊗	Palermo, Divisione Ematologia	–	1	1	6	8	R. Scimè
°	Firenze, Cattedra di Ematologia	3	3	–	2	8	A. Bosi
⊗	Pescara, Divisione Ematologia	4	1	2	–	7	P. Di Bartolomeo
*	Pisa, Clinica Pediatrica	2	–	3	1	6	C. Favre
°	Bologna – Cattedra Ematologia	1	1	–	4	6	G. Bandini
*	Trieste, Clinica Pediatrica	5	1	–	–	6	M. Andolina
°	Genova, S.Martino - Divisione Ematologia	1	2	–	2	5	A. Bacigalupo
°	Pavia, Policlinico S.Matteo Div. Ematologia	–	2	–	2	4	E.P. Alessandrino
°	Verona, Cattedra Ematologia	–	1	2	1	4	F. Benedetti
⊗	Vicenza, Divisione Ematoloiga	1	2	-	-	3	F. Rodeghiero
⊗	Udine, Cattedra di Ematologia	–	1	-	2	3	R. Fanin
*	Cagliari, Clinica Pediatrica	–	-	3	-	3	F. Argiolu
°	Milano, Ospedale Niguarda-Div Ematologia	–	1	–	1	2	P. Marenco
°	Torino, Divisione Ematologia	–	–	–	2	2	M. Falda
°	Milano, IRCCS Osp. Maggiore Centro TMO	–	–	–	2	2	D. Soligo
⊗	Reggio Calabria, CTMO Div.e Ematologia	–	–	2	–	2	P. Iacopino
⊗	Bergamo, Divisione Ematologia	–	–	–	1	1	A. Rambaldi
°	Perugia, Cattedra Ematologia	–	1	–	–	1	F. Aversa
°	Napoli,Divisione Ematologia	–	–	1	–	1	B. Rotolo
°	Roma, Ospedale S.Eugenio - Div. Ematologia	–	–	1	–	1	I. Majolino
°	Catania, Istituto Ematologia	–	–	–	1	1	G. Milone
	TOTAL	210	43	212	55	520	

**Exclusively Pediatric AIEOP centers; ⊗AIEOP centers in adult institutions; °non AIEOP adult institutions.*

Allogeneic transplant early in the course of disease should be the approach of choice for all patients less than 19 years old.

The results we obtained in patients with inborn errors are similar to those of other series,[16-18] and confirm our previous reports.[19-20] Severe combined immunodeficiency, hemophagocytic lymphohistiocytosis, Wiskott-Aldrich's disease, storage disorders and several others congenital diseases are absolute indications for UD BMT. TRM and graft failure are the main obstacles to the success of this procedure in patients with Fanconi's anemia and osteopetrosis. Long-term follow-up of children with storage disorders suggests that following BMT the loss of intelligence continues despite persistent engraftment and normalization of enzymatic activity. Therefore, early diagnosis is very important if an UD BMT is to be attempted.[21]

In a previous report we showed that rejection was the main problem for thalassemic patients who undergo UD BMT. The incidence of rejection seems to have decreased with the introduction of

thiotepa in the conditioning regimen of more recent patients (*data not shown*). The indication for UD BMT is still a crucial problem in the therapeutic decision for thalassemic patients lacking a matched sibling. In these cases extensive DNA study of class II antigens is recommended and the patient should proceed to transplant only if a fully matched donor is available.

In conclusion, our experience shows that the number of children with a suitable matched UD has increased and that the outcome has improved over the years. Nowadays, the number of children undergoing an UD BMT is higher than their number of children given a BMT from a matched sibling in several institutions, and the results that are achieved are comparable.

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Unrelated bone marrow transplantation in thalassemia. The experience of the Italian Bone Marrow Transplant Group (GITMO)

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Background and Objectives. Allogeneic bone marrow transplantation (BMT) is a widely accepted therapeutic approach in homozygous β-thalassemia. However, the majority of patients do not have a genotypically identical donor within the family. This prompted us to conduct a pilot study to investigate the feasibility of matched unrelated bone marrow transplantation in thalassemia. The major drawback was the high risk of immunologic and transplant-related complications, mainly graft-versus-host disease (GvHD) and graft failure.

Design and Methods. Our aim was to reduce this risk through careful selection of donor/recipient pairs. HLA haplotypes that show a high linkage disequilibrium among their class I, class II and class III alleles are considered extended or ancestral haplotypes.

Results. These haplotypes are conserved and can be shared by apparently unrelated individuals. Our study shows that matching for these haplotypes significantly improves the outcome of unrelated bone marrow transplantation in thalassemia. In fact, results were comparable to those obtained in transplants using HLA-identical family donors.

Interpretation and Conclusions. Better results were obtained in patients with lesser iron overload and when the donor shared an identity for the DPB1 alleles.

Key words: unrelated BMT, thalassemia, extended haplotypes, DPB1 alleles.

In Western countries, more than 70% of patients with homozygous β-thalassemia lack an HLA-identical family donor. Until recently this left them with no option other than life-long transfusion and iron-chelating therapy. Over the past ten years, there has been a steady increase in the number of bone marrow transplants (BMTs) from unrelated donors.[1] At first, results were unsatisfactory with a high risk of transplant-related complications, particularly acute and chronic graft-versus-host disease (GvHD) and graft failure. These complications are likely to be the consequence of HLA differences not revealed by previous HLA typing techniques.[2] The introduction of high resolution molecular techniques for histocompatibility testing has markedly improved the outcome of unrelated transplants with results comparable to those obtained in transplants using HLA-matched family donors.[3] An important challenge was to achieve similar results in thalassemic patients.

In HLA-matched unrelated individuals, the entire structure of an HLA extended or ancestral haplotype (EH) is generally identical except for rare variations at the centromeric and telomeric extremities. Two unrelated individuals who share two extended haplotypes are highly likely to be identical, not only for the routinely tested HLA class I, II and class III genes, but for the entire MHC region where there are many other genes that have an important role in antigen presentation and immune response.[4] Several mechanisms, including selection pressure, recombination suppression and preferential transmission, may explain the conservation and frequency of extended or ancestral haplotypes in different populations.[5] Based on the foregoing, we conducted a pilot study aimed at

exploring both the feasibility of BMT from a marrow unrelated donor (MUD) in thalassemia and the possibility of reducing the risk of immunologic complications by selecting donor/recipient pairs sharing extended haplotypes or parts of them.

Design and Methods

From November 1992 to February 2002, 43 patients with thalassemia major were enrolled into this study by six BMT Centers in Italy. Twenty of the patients were females and 23 males, their ages ranging from 2-28 years (median 14). Out of 43 patients examined, 19 were assigned to low and intermediate risk classes, and 24 to the high risk class. Alleles at the HLA-A, B, Cw, DRB1, DRB3, DRB4, DRB5, DQA1, DQB1 and DPB1 loci were identified by PCR-SSP (Dynal, Oslo, Norway) and sequence-based typing. Amplification and sequencing of HLA class I and class II genes were performed using standard big dye terminator cycle-sequencing chemistry supplied with the ABI sequencing kit. Reactions were analyzed on an Applied Biosystem 310 Automated DNA sequencer. Alleles were assigned according to DNA sequences published by the *Nomenclature for the factors of HLA system* Committee.

In our study, HLA extended haplotypes were identified and defined by referring to the data provided by Rendine *et al.* and Contu *et al.* for the Italian population and to data from the 10th and 11th International Histocompatibility Workshops and the National Marrow Donor Program donor registry for other populations.[6-8]

Thirty-eight bone marrow donors were identified within the *Italian Bone Marrow Donor Registry*, another 3 were found in the *German National Bone Marrow Donor Registry*, one was found in the *French Bone Marrow Donor Registry*, and one in the *National Marrow Donor Program Donor Registry* of the USA. For 28 donors, at least one informative family member was typed for HLA haplotype deduction. In the remaining 15 cases, the haplotypes were assigned on the basis of the presence of at least one extended haplotype well-defined in the population. Six patients were transplanted after a preparative regimen including busulfan (BU) 14 mg/kg and cyclophosphamide (CY) 200/160/120 mg/kg. As 2 of these 6 patients did not have sustained engraftment, in the remaining 37 patients the conditioning regimen was modified as follows: BU 14, thiotepa (TT) 10 mg/kg and CY 200 for 17 patients (low and intermediate risk classes); BU 14, TT 10 and CY 160 for 6 patients aged less than 16 (high risk class); BU 14, TT 10 and CY 120 for 14 patients aged more than 16 years old (high risk class: adults). The median bone marrow nucleated cell dose was 3.6×10^8/kg of recipient weight (range 1.8-11.6). All patients received cyclosporine (CSP) and short-term methotrexate (MTX) for GvHD prophylaxis. Acute and chronic GvHD were graded according to the Seattle criteria. Chimerism was documented by *in situ* Y chromosome hybridization of either bone marrow or blood samples in sex-mismatched donor/recipient pairs, by analysis of variable number of tandem repeat (VNTR) polymorphisms and by microsatellite analysis of bone marrow and/or blood samples in the case of sex-matched pairs. For continuous variables with a symmetric distribution, the results are expressed as medians and ranges. Comparison between groups was performed by Fisher's exact test. Survival probability was estimated by the product-limit method of Kaplan and Meier.

Results and Discussion

Out of 43 donor/recipient pairs, 33 were completely identical for HLA-A, B, C, DRB1, DRB3, DRB4, DRB5, DQA1 and DQB1 loci. Seven pairs were completely identical for two extended haplotypes and 20 pairs shared one extended haplotype. Although the remaining 16 pairs did not share complete extended haplotypes, family segregation analysis performed in five cases in both donor and recipient showed haplotype identity in 3 cases. Only in one case was haplotype identity lacking.

In 30 cases (69.8%) the transplant was successful with complete allogeneic reconstitution. Five patients (11.6%) rejected the donor marrow and eight patients (18.6%) died from transplant-related complications.

None of the 7 recipients who shared two HLA EH and had sustained donor engraftment developed grade II-IV acute GvHD, while in the remaining 30 evaluable patients who shared either a single or no EH, the overall occurrence of acute GvHD was 52% (p=0.05). A significant reduction of the incidence of acute GvHD was observed in the group of patients who shared an identity for both DPB1 alleles with their donors, compared to in the group of patients who received bone marrow from a donor mismatched for either 1 or 2 DPB1 alleles (21% vs 61%, p =0.05). Moreover, the risk of acute GvHD was significantly increased (p = 0.01) in patients with an HLA class I minor-mismatch and one or two differences at the DPB1 locus compared to patients sharing at least one extended haplotype

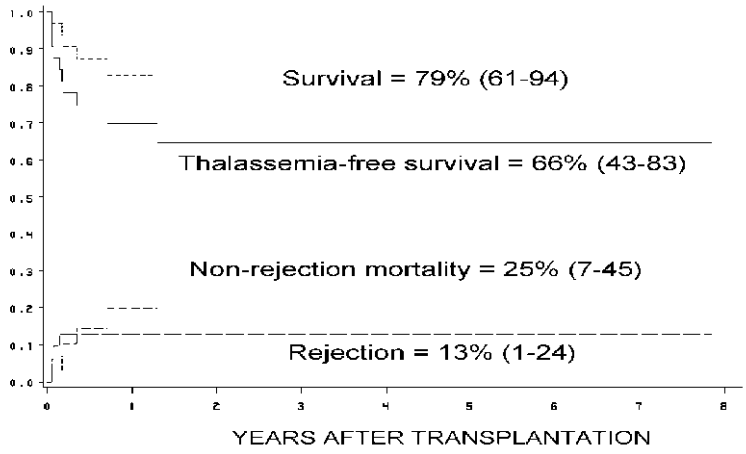

Figure 1. Kaplan-Meier probabilities of survival, thalassemia-free survival, non-rejection mortality and rejections for 41 thalassemia patients transplanted from HLA-matched unrelated donors (between parentheses, 95% confidence limits at two years).

and DPB1 identity with their donors (100% vs. 18%, respectively). In our series of 43 consecutive thalassemia patients, rejection and mortality rates were 11.6% and 18.6%, respectively. Sixty-nine percent of our patients are alive with sustained engraftment of donor hematopoiesis, this leading to a projected thalassemia-free survival of 66% (Figure 1). Overall survival and thalassemia-free survival (94.7% and 84.2%, respectively) in the 19 patients of class I and class II risk groups were comparable to those obtained in transplants from an HLA-identical family donor. The remarkable stability of the extended haplotypes[4] and the data deriving from MLC studies[9] suggest that two unrelated individuals sharing two HLA extended haplotypes are nearly always practically HLA-genoidentical, just as if they had inherited the HLA haplotypes from the same parents. Therefore, it is reasonable to hypothesize that the histocompatibility differences between a pair of HLA-genoidentical siblings and a pair of unrelated individuals sharing two extended haplotypes exclusively reside in minor histocompatibility antigens (mHAg) located outside the HLA region. Moreover, haplotype matching, even when it is not for complete extended haplotypes, makes it possible to include parts of them (telomeric or centromeric portion of extended haplotypes) that are common in populations worldwide.[10] The relatively low incidence of acute and chronic GvHD (44% and 28%, respectively) obtained in our study highlights the importance of a careful immunogenetic selection of donor-recipient pairs. So far, it is quite difficult to differentiate the role of DP[11] molecules from that of other HLA molecules as this would require the availability of genoidentical donor/recipient pairs different at the DP locus for a rare event of crossing-over. Alternatively, an optimal model for this type of evaluation is represented by unrelated donor/recipient pairs different at the DP locus but sharing two extended haplotypes. In our study, the incidence of immunologic complications was significantly reduced in DPB1-matched recipients.

Our results show that BMT from unrelated donors, especially when identical for at least one extended haplotype, may offer a probability of success comparable to that offered by transplants using HLA-identical family donors. It is, therefore, reasonable to consider this type of transplant as an acceptable therapeutic approach in thalassemia, at least for patients who are not fully compliant with conventional treatment and do not yet show irreversible severe complications of iron overload, provided that a careful immunogenetic selection of marrow donors is made.

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The therapeutic role of dendritic cells in cancer immunotherapy

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Dendritic cells (DC) are professional antigen-presenting cells (APC) specialized in capturing and processing antigens into peptide fragments that bind to major histocompatibility complex (MHC) molecules. DC are the most potent stimulators of T-cell responses and they are unique as they stimulate not only memory but also naive T-lymphocytes. Thus, DC play a pivotal role (*nature adjuvants*) for the induction of B- and T-cell-mediated immune responses.[1] A growing body of evidence supports the role of DC for stimulating specific anti-tumor immunity in animal models and humans. Moreover, DC isolated from cancer patients have shown a functional deficit in stimulating allogeneic T-cells and, more important, in presenting tumor antigen to autologous T-cells.[2-4] The defective function of DC has been shown to be dependent on the production by tumor cells of cytokines, such as vascular endothelial growth factor (VEGF)[2] or interleukin (IL)-6,[4] that inhibit the differentiation of hematopoietic progenitors to the DC lineage. These findings further underscore the role of DC in cancer immunology and provide an explanation for the failure of the immune system to control tumor growth once the function of DC is impaired. In addition to their activity as stimulators of T-cell immunity, DC are critical for the induction of central and peripheral tolerance to self-antigen.[1]

Biological characterization of DC

DC are widely distributed in the body and are particulary abundant in tissues that interface the environment (i.e. Langerhans' cells in the skin and mucous membranes) and in lymphoid organs (interdigitating DC) where they act as *sentinels* for incoming pathogens. Inflammatory signals such as tumor necrosis factor (TNF)-α and IL-1β as well as bacteria, bacterial products (LPS) and viruses induce migration of antigen-loaded DC from the peripheral tissues to secondary lymphoid organs. During migration, DC mature and upregulate MHC, adhesion and co-stimulatory molecules, so that their ability to prime T-cells is markedly improved.[1]

The functional activity of DC derives from a number of properties of these cells: their dendritic shape, along with the high level of expression of certain adhesion molecules and integrins (LFA-3, ICAM-1, ICAM-3), increases the area of contact with the effector cells of the immune system.[5] DC strongly express the HLA class II molecules –RD, –DQ and –DP and co-stimulatory molecules (CD80, CD86 and CD40) which activate their ligands on T-cells (CD28, CTLA-4 and CD40L), thus providing the *second signal* strictly necessary to induce a proliferative response, rather than tolerance, upon antigen recognition. In addition, DC produce a number of cytokines including IL-12 which promote a cytotoxic immune response by inducing the differentiation of Th0 cells to interferon (IFN)-γ and IL-2-producing Th1 cells.[6,7] It has recently been demonstrated that upon antigen recognition, $CD4^+$ cells activate DC via CD40-CD40L interaction and *activated* DC are then able to trigger the cytotoxic activity of $CD8^+$ cells.[8-10]

However, DC are present in peripheral tissues in an *immature state* unable to prime T-cells. At this stage of differentiation, they can very efficiently take up soluble antigens, particles and microorganisms by phagocytosis, macropinocytosis or by the macrophage mannose receptor, Fcγ and Fcε receptors whereas they lack all the accessory signals for T-cell activation. Antigen uptake induces DC to maturation by upregulating MHC and co-stimulatory molecules as well as DC-associated antigen (e.g. CD83 and p55) whereas the capacity of capturing and processing antigen is lost. However, full activation of DC is dependent upon the contact with T-cells by the CD40-CD40L interaction which induces the production of IL-12. Thus, the key functions of DC (antigen uptake, T-cell

stimulation) are strictly segregated to subsequent stages of differentiation. As mentioned above, IL-10,[11] VEGF[2] and IL-6,[4] secreted by cancer cells, prevent the maturation of DC thus inhibiting the efficient priming of T-cells.

Different strategies for the generation of DC ex vivo

Circulating CD14+ monocytes represent the most readily available source of DC if incubated with appropriate cytokines such as granulocyte/macrophage colony-stimulating factor (GM-CSF), IL-4 and TNF-α.[12,13] Moreover, DC precursors have been isolated within the CD34+ cell fraction in bone marrow, cord blood and steady state or mobilized peripheral blood (PB).[14] Also in this case the differentiation of CD34+ cells into fully functional DC is strictly dependent upon stimulation with certain cytokines such as GM-CSF, TNF-α, SCF, FLT3-L and IL-4. In this model, the early-acting growth factors SCF and FLT3-L expand the number of CD34+ cells which then respond to GM-CSF and TNF-α,[15] Recently, the phenotypic and functional characteristics of DC derived from CD34+ cells mobilized into PB or from BM progenitors have been formally compared.[16] The published results indicate that G-CSF mobilizes DC precursors with an increased frequency and a higher proliferative capacity than their bone marrow counterparts. This finding translates into a higher number of mature DC generated in liquid culture. Despite pretreatment with G-CSF, these cells maintain the same functional capacity of stimulating allogeneic T-cells as marrow-derived DC. CD34+ cell-derived DC are also capable of processing and presenting soluble antigen to autologous T-cells for both primary and secondary immune responses. However, in cancer patients the proliferative potential of CD34+ cells is impaired. In fact, comparative studies performed in multiple myeloma patients demonstrated a higher yield of mature and fully functional DC when CD14+ monocytes were cultured as compared with CD34+ cells.[17] GM-CSF and IL-4 induce the differentiation of non-proliferating CD14+ monocytes to immature DC with a low level of expression of CD83 and p55 antigen and largely incapable of priming naive T-cells. These immature DC are not fully differentiated and revert to an adherent state if the cytokines are removed from the culture medium.[18] The addition of inflammatory cytokines such as TNF-α, IL-1β or PGE_2 for 1-2 days to the medium containing GM-CSF and IL-4 promotes the maturation of DC and increases the ability to stimulate T-cells. Thus, immature DC generated from CD14+ cells in the presence of GM-CSF and IL-4 are well equipped for capturing and processing soluble TAA. However, they do require a further maturation stimulus to exert their stimulatory effect on T-cells. Immature DC are the ideal targets for genetic manipulation using viral or bacterial vectors which infect non-replicating cells (*see below*). In this case, the modified pathogens can, by themselves, induce the full maturation of DC. In alternative, mature DC could be used in vaccination protocols involving TA peptides as DC also prime T-cells to foreign antigen that bind directly to MHC molecules without prior processing.[19]

Very recent data indicate the mobilization of large numbers of DC precursors by GM-CSF[20] and FLT-3L.[21] However, it remains to be tested whether circulating DC are as efficient as monocyte-derived DC (Mo-DC) in stimulating a specific CTL response to tumor antigen. In this regard, PB DC in multiple myeloma are defective in presenting the patient-specific tumor idiotype to autologous T-cells whereas *ex vivo* generated Mo-DC are fully functional APC.[4] Conversely, in the case of myeloproliferative disorders, the efficient generation of leukemic DC carrying the tumor-specific genetic alteration and their use to induce leukemia-specific CTL have been reported.[22,23]

Delivery of TAA to DC

Several methods for the efficient delivery of TAA to DC have been described so far.[1] This is based on the finding that tumor cells are often poorly immunogenic due to the lack of T-cell recognition, activation and co-stimulation typical of professional APC. To this end, murine DC have been fused with the carcinoma cell line MC38 to provide tumor cells with the functional characteristics of DC.[24] The fusion cells showed all the phenotypic features of DC and were shown to be capable of preventing tumor growth when the mice were challenged with the cell line. Moreover, treatment with fusion cells induced the rejection of pulmonary metastases. This strategy has recently been applied to a clinical trial of renal cell carcinoma patients.[25]

Several TA peptides which are presented to T-cells in association with HLA class I molecules have recently been identified and proved to be useful in stimulating an autologous CTL response *in vitro* and *in vivo*.[26,27] However, pulsing DC with peptides may not be optimal for clinical application because of the strict MHC restriction of the immune response and their limited stability. In addition, pulsing with peptides may not induce a T-cell

response directed toward tumor cells expressing the relevant antigen. An attractive alternative is the use of unfractionated tumor-derived proteins, when available, apoptotic cells[28] or tumor lysates. In the last case the obvious disadvantage is the possibility of inducing immune responses against self-antigen expressed in tissues other than tumor cells.

A further possibility is the transduction of DC with expression vectors encoding for TAA genes. DC can be engineered by various means which differ with respect to the capacity of targeting quiescent cells, stable integration in the genome, infection efficiency and stimulation of anti-tumor immunity. Retrovirally-transduced DC costitutively express the relevant sequence and are potent stimulators of a specific T-cell response.[29] However, retroviral vectors have a relatively low efficiency of transduction, they can only infect actively replicating cells and carry the theoretical risk of oncogenic trasformation of target cells. Conversely, adenoviruses infect both quiescent and proliferating cells and do not integrate into DNA. Moreover, supernatants with a high titer of the virus can be easily obtained. Recently, DC have been transduced with adenovirus combined with cationic liposomes showing an infection efficiency close to 100%.[30] The major limitation to the clinical use of adenoviruses is their high immunogenicity which induces the production of neutralizing antibodies and the rapid development of CTL directed to infected cells.

Two phase I clinical trials have been recently conducted to assess the safety of vaccinia virus vectors engineered to express human papilloma virus (HPV) and carcinogenic antigen (CEA) genes and the capacity of stimulating an immune response.[31,32] More recently, maturation of DC and efficient induction of both CD4 and CD8 T-cell activation have been induced by infection with bacterial vectors.[33] As a result, a model antigen (ovalbumin), expressed on the surface of recombinant *Streptococcus gordonii*, is processed and presented on MHC class I molecules 10^6 times more efficiently than soluble OVA protein. Therefore, bacterial vectors are a potentially useful means of delivering exogenous antigen to DC for stimulating a tumor-specific CTL response *in vitro*[33] and *in vivo*.[34] A different approach has been taken by Boczkowsky *et al*.[35] who transfected DC with the total RNA extracted from tumor cells and combined with cationic lipid to enhance the infection efficiency. Similarly to the use of tumor lysates, this strategy applies to those situations in which a tumor-specific antigenic marker is lacking whereas the major concern is the increased risk of autoimmune reactivity.

DC for cellular immunotherapy

The central role of DC in stimulating a tumor-specific immune response has been well established by *in vitro* and *in vivo* animal models.[26,27] In humans, initial studies were performed in melanoma patients using DC pulsed with MAGE peptide.[36,37] The infusion of loaded DC induced the migration of MAGE-specific CTL to the site of injection and increased the frequency of circulating tumor-specific CTL. More recently, advance stage melanoma patients have been treated with intranodal injection of peptides or tumor lysate-pulsed DC according to the HLA profile of the patient.[38] The authors reported the stimulation of a peptide-specific T-cell response in all cases. Moreover, in 5/16 patients an objective clinical response was observed. In this study, DC were generated *ex vivo* from monocyte precursors in the presence of IL-4 and GM-CSF and directly injected into an inguinal lymph node to reach T-cell rich areas. A similar strategy to target MAGE-3A1 antigen has been applied for melanoma patients with metastatic disease.[39]

Tumor-specific peptides (fragments of prostate specific antigen, PSA) have also been used to pulse autologous DC in prostate cancer patients refractory to hormone-therapy.[40] Seven out of 51 patients showed a partial response while none of the patients in the control group, injected with peptides alone, showed any clinical benefit. In B-cell malignancies, the patient-specific idiotype gene sequence and its protein product represent the optimal targets for vaccination strategies.[41] Hsu *et al.* have reported on 4 low-grade non-Hodgkin's lymphoma (NHL) patients resistant to conventional chemotherapy or relapsed with DC pulsed with the idiotype as soluble antigen.[42] A tumor-specific T-cell response was observed in all cases, coupled, in one case with the regression of tumor burden. Very recently, 35 additional patients have been reported and a tumor-specific cellular response has been found in 17 individuals.[43] The same strategy of targeting the idiotype has been proposed by the same group for inducing a T-cell immune response in multiple myeloma patients.[44] In these trials, DC were freshly isolated from the PB by subsequent enrichment steps and were reinfused intravenously. However, this approach raises concerns on both the efficacy of PB DC of stimulating efficiently T-cells and the capacity of idiotype-loaded APC to reach secondary lymphoid organs to prime T-cells, escaping the entrapment of the pulmonary apparatus.

In conclusion, the clinical data available so far have provided the *proof of principle* that autologous DC generated *ex vivo* and reinfused into cancer patients are effective in stimulating an antitumor immune response. Although, it remains to be determined which of the several strategies proposed for cellular immunotherapy is the most efficient and it may well be that different tumors require different approaches, DC-based immunotherapy holds promises of exerting a potent antitumor effect in humans.

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