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Clinical use of allogeneic hematopoietic stem cells from sources other than bone marrow

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Abstract

Background and Objective. **Peripheral blood stem cells (PBSC) are being increasingly used as an alternative to conventional allogeneic bone marrow (BM) transplantation. This has prompted the Working Group on CD34-Positive Hematopoietic Cells to evaluate current utilization of allogeneic PBSC in clinical hematology.**

Evidence and Information Sources. **The method employed for preparing this review was that of informal consensus development. Members of the Working Group met three times, and the participants at these meetings examined a list of problems previously prepared by the chairman. They discussed the single points in order to reach an agreement on different opinions and eventually approved the final manuscript. Some of the authors of the present review have been working in the field of stem cell transplantation and have contributed original papers in peer-reviewed journals. In addition, the material examined in the present review includes articles and abstracts published in journals covered by the Science Citation Index® and Medline®.**

State of the Art. **Review of the current literature shows that unmanipulated allogeneic PBSC give prompt and stable engraftment in HLA-identical sibling recipients. Despite the much higher number of T-cells infused, the incidence and severity of acute GVHD after PBSC transplant seems comparable to that observed with bone marrow (BM) cells. In comparison to the latter, PBSC probably ensure faster immunologic reconstitution in the early post-transplant period. Controversial results on the incidence and severity of acute-GVHD have been reported when CD34+ selection methods are used. Prospective randomized trials are underway to compare the results of PBSC and BM allogeneic transplantation. In mismatched family donor transplants, T-cell depleted PBSC successfully engraft immune-myeloablated recipients through a mega-cell-dose effect able to overcome the HLA barrier. Experience with PBSC in the context of unrelated donor transplants is currently anecdotal and prospective trials should be completed before that practice becomes routine. Finally, there is also limited evidence that, following induction chemotherapy, the addition of PBSC to donor lymphocyte infusion (DLI) for treatment of leukemia relapse after BMT may improve the safety and effectiveness of DLI itself. Concerning cord blood (CB) transplants, the most interesting aspects are the ease of CB collection and storage, the low risk of viral contamination and the low immune reactivity of CB cells. This last property has its clinical counterpart in an apparently reduced incidence and severity of acute GVHD both in sibling and unrelated CB transplants, probably making the level of donor/recipient HLA disparity acceptable a greater degree with respect to what is required for transplants from other sources.**

Key words: hematopoietic stem cells, bone marrow, cord blood, peripheral blood, allogeneic transplantation, graft-versus-host disease

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In the field of allogeneic transplantation the use of alternative sources of stem cells, namely peripheral blood stem cells (PBSC)[1] and placental cord blood (CB) stem cells,[2] is rapidly expanding. The European Group for Blood and Marrow Transplantation (EBMT) registered only 12 allogeneic PBSC transplants in 1993, but this number increased to 180 in 1994 and to 571 in 1995.[3] Concerning cord blood (CB) transplants, following initial attempts[4,5] considerable experience has now been achieved in the USA and Europe so that this modality is entering a phase of extensive clinical application, with hundreds of procedures registered both from sibling and unrelated donors,[6,7] The ease of collection and storage of CB stem cells and the apparent tolerance-inducing property of

CB $CD8^+$ suppressor cells[8] are the most interesting aspects of this latter source.

Stem cells in peripheral blood and CB both possess a *migratory* status and differ in part from those found in bone marrow with respect to their biological and functional properties. However, while PBSC are envisaged as a means of improving results by increasing the number of cells available, placental CB stem cells open the realistic perspective of increasing the number of transplants thanks to the availability of thousands of cord samples for patients who lack a compatible donor among family members.

This search for new stem cell sources also arises from the fact that allogeneic bone marrow transplantation still carries a high procedure-related mortality and disease recurrence rate. Cell dose has an influence on engraftment and chance of survival. In a retrospective study Bacigalupo *et al*.[9] showed that patients with hematologic malignancies who receive allogeneic bone marrow grafts with higher CFU-GM numbers have significantly higher platelet counts on day +80 and a lower mortality rate than those who receive fewer CFU-GM. The effect of CMV infection on platelet counts also appears to be less pronounced when the number of progenitor cells is higher. The use of more hematopoietic progenitors would then result in improved transplant outcome.

The growing interest in allogeneic PBSC induced the *Italian Bone Marrow Transplant Group* (GITMO) to draw up a list of recommendations that were originally published in 1995[10] and recently revised in light of the increasing experience gained worldwide during the last two years.[11] Moreover, in a previous issue of this Journal[12] a review article analyzed the biological and technical aspects of PB and CB stem cells. The key aspects dealt with were the mobilization and collection methods, the capacity for stable hemopoietic reconstitution, kinetic characteristics and immunological features.

Historical background

Interest in allogeneic transplantation of PBSC began thirty years ago. In the late sixties, based on an earlier demonstration that autologous PBSC were capable of restoring irradiation-myeloablated hematopoiesis,[13-15] the Seattle group reported the first successful attempts at allogeneic PBSC transplantation in dogs[16,17] and non-human primates.[18] However, due to the high GVHD incidence, those experiments were unable to demonstrate long-term stability of the graft. Only a decade later did purification of PBSC and application of cytogenetic methods allow a group of German investigators[19,20] to document in dogs the stability of donor-derived hemopoietic function for more than ten years after PBSC allogeneic transplantation.

A key issue at that time was the low number of progenitors in steady-phase peripheral blood, and clinical application of PBSC was limited to autologous transplantation in CML,[21] where hematopoietic progenitors, mostly of the leukemic counterpart, circulate in high numbers and can easily be collected by apheresis without any prior stimulation. A further step was the demonstration that the PBSC level increases dramatically during the post-chemotherapy recovery phase;[22] however, it was the advent of G-CSF and GM-CSF that provided the rapid expansion of PBSC technology and led to their use in allogeneic transplantation as well. The pioneer work of Socinski *et al*.[23] and Gianni *et al*.[24] established the ability of hematopoietic growth factors to expand the circulating progenitor cell pool either when used alone or in conjunction with chemotherapy. However, transferring growth-factor PBSC mobilization strategy from autograft patients to normal donors took some years. In fact, the safety of growth factors and the clinical applicability of allogeneic PBSC in terms of GVHD incidence and long-term engraftment represented serious reasons for caution. Clinical PBSC allogeneic transplantation began in 1989, when Kessinger *et al*.[25] reported the first attempt in an HLA-matched recipient with ALL. The patient was an 18-year-old man in third remission after CNS and testicular relapse. His sibling female donor preferred to donate PBSC rather than bone marrow, and she underwent 10 apheretic procedures without any mobilization treatment. The apheresis product was T-depleted by sheep erythrocyte rosetting and infused after conditioning with high-dose Ara-C and TBI. The patient achieved full donor engraftment as demonstrated by cytogenetic studies but died on day +32, and sustained engraftment could not be demonstrated.

Four years later, in 1993, Russell *et al*.[26] reported another transplant in a patient whose sibling donor presented an increased risk of complications from anesthesia. In this case, 10 mg/kg/day of G-CSF were given to mobilize PBSC. The cells were collected at two leukaphereses containing 36.8×10^4/kg CFU-GM and infused without any prior manipulation. Engraftment occurred rapidly and GVHD did not develop despite the high T-cell content of the graft sample. The same year, a group of investigators from Kiel University[27] also successfully employed allogeneic PBSC. A 47-year-old AML patient who failed to engraft after bone marrow transplantation from an HLA-identical sibling donor was infused with the unmanipulated product of 3 leukaphereses performed after treating the donor with 6 mg/kg/day G-CSF. Engraftment occurred on day +14, with moderate acute GVHD that responded to immunosuppression starting on day +18. Restriction fragment length polymorphism (RFLP) typing demonstrated full donor engraftment

up to 60 days following transplantation.

Another important step was the five PBSC transplants from syngeneic donors performed in Seattle and reported in 1993:[28] with a median of 9.6×10^6/kg CD34$^+$ cells infused, the patients engrafted 0.5×10^9/L granulocytes on day 13 and 20×10^9/L platelets on day 10. In 1995 three separate reports appeared in the same issue of Blood, one from Seattle,[29] a second from Houston[30] and the other from Kiel;[31] a total of 25 patients were allografted with PBSC from their HLA-identical sibling donors. Acute GVHD was apparently not increased in those series. Molecular analysis of engraftment[30,31] furnished definitive proof of the experimental data[20] suggesting that allogeneic PBSC contain true long-term repopulating stem cells. The high engraftment potential of PBSC was exploited by the Perugia team[33] to successfully transplant leukemia patients from their haploidentical, *three-loci-incompatible* family donors through T-cell depletion. Finally, Ringdén *et al*.[34] recently reported the use of allogeneic PBSC in selected unrelated donor transplants.

The kinetics of PBSC under cytokine mobilization was extensively analyzed in a previous review published in this Journal.[12] PBSC mobilization in healthy donors is best accomplished with G-CSF. The aspect of donor safety was analyzed in a cooperative GITMO study[35] in which short-term side effects were shown to be minimal. Ten µg/kg/day of G-CSF for 5 days enabled the collection of $>4\times10^6$/kg CD34$^+$ cells with two aphereses in 85% of donors. Variations in blood counts included a sharp elevation of WBC and CD34$^+$ cells and a moderate transitory thrombocytopenia. One problem, however, is the lack of data on the late effects of G-CSF. At the Geneva conference on allogeneic PBSC, Hasenclever and Sextro[36] presented a feasibility study of long-term risk analysis. In order to demonstrate a tenfold increase in leukemia risk, more than 2000 healthy PBSC donors would have to be followed for over 10 years. A control group of BMT donors of equal size would also be necessary. Such a study could only be carried out on a multinational basis.

Transplantation of allogeneic PBSC from HLA-identical siblings

Conditioning regimens and GVHD prophylaxis

Conditioning regimens employed in PBSC transplantation are the same as those used for bone marrow transplantation (BMT). As listed in Table 1, the majority of patients received CY-TBI or BU-CY with CY at 120 or 200 mg/kg. Indeed, 33.8% and 24.5% of reported patients were conditioned with CY-TBI and BU-CY, respectively. Analogously to BM transplantation, patients with SAA received cyclophosphamide alone or in association with ATG.

Recently, some innovative regimens have been developed in order to: i) increase antitumor activity; ii) reduce treatment-related toxicity. For instance, high dose Ara-C or VP16 has been employed for patients with more advanced disease. Others considered thiotepa, a potent myeloablative drug first introduced in conditioning by the Perugia group.[37] This latter compound was used along with classical BU-CY2 in a large series at the M.D. Anderson Cancer Center[38,39] or was associated with cyclophosphamide.[40] In this study, thiotepa was introduced in the hope of reducing the liver toxicity of busulfan.

It is interesting to note the recent introduction of fludarabine, a purine analogue initially proposed at conventional dosage by the M.D. Anderson group[41] and at a higher dose by the Perugia group in HLA-mismatched transplants.[42] Fludarabine has been associated with several other drugs or drug combinations including cyclophosphamide plus cis-platinum, high-dose (HD) Ara-C, idarubicine plus Ara-C or melphalan.[41,43] These fludarabine containing regimens were followed by full engraftment with complete donor chimerism in the absence of severe aplasia. Basically, these new regimens allow allograft even in older or medically infirm patients, since they reduce the toxicity but still maintain an effective graft versus leukemia reaction. For the same reason Adkins *et al.*[44] combined low-dose TBI (550 rad) at a high dose rate (30 cGy/min) with cyclophosphamide 120 mg and methylprednisolone 2 g over two days.

A non-myeloablative regimen with busulfan and methylprednisolone combined with immunosuppression with the CD3 monoclonal antibody was used by Tan *et al.*,[45] while Slavin *et al.*[46] employed fludarabine and ATG as intensive immunosuppression associated with busulfan at 4 mg/kg/day over 2 days. Although these regimens are not specifically designed for PBSC transplantation, the high number of inoculated PB stem cells overcomes graft rejection, favoring rapid and stable chimerism.

Table 1. Conditioning regimens and GVHD prophylaxis: 301 patients.

Regimens	*No.*	*%*	*GVHD prophylaxis*	*No.*	*%*
CYTBI	102	33.8%	CsA-MTX	150	49.8%
BUCY	68	24.5%	CsA-MP	82	21.2%
TioBUCY	67	22.2%	FK506-MTX	7	2.3%
VP16TBI	10	3.3%	FK506-MP	44	14.6%
TioCY	8	2.6%	CsA	15	2.9%
CYATG	3	0.9%	MTX	1	0.3%
Others	43	16%	Others	2	0.6%

The large amount of stem cells in the inoculum might explain the full engraftment observed in a patient who could not complete the whole regimen and received busulfan alone as preparation.[47]

The large number of $CD3^{+ve}$ cells present in the PB-derived inoculum has raised some concerns about the severity of GVHD following PBSC allograft. However, GVHD prophylaxis has not been substantially modified from the standard regimens used for bone marrow transplants. Cyclosporin A (CsA) has been used alone (2.9%) or in association with either methotrexate (MTX) (49.8%) or methylprednisolone (MP) (21.2%) in 71% of patients (Table 1).

New immunosuppressive regimens including tacrolimus (FK506) in association with methylprednisolone, methotrexate[39] or monoclonal antibodies[48] have been explored in 17% of cases.

Finally, some centers have developed techniques for *in vitro* stem cell enrichment. However, using Ceprate-cell separation, a 2-3 log depletion of T-lymphocytes is not enough to avoid the risk of GVHD and further immunosuppression is generally required.

In some institutions cryopreservation of collected PBSC is preferred to freshly collected material for several reasons. First, cryopreservation allows precise evaluation of the hemopoietic progenitor content in the harvested material. Second, the allograft may be scheduled at the proper time once adequate quantities of PBSC are collected. Finally, although still unproven, a reduced risk of acute GVHD in patients transplanted with cryopreserved BM cells has been suggested.[49]

Engraftment

Engraftment kinetics following PBSC allograft has been extensively investigated. None of the studies include patients dying before day 21. The median time to reach an absolute neutrophil count(ANC) above 0.5×10^9/L ranges between 10-16 days. The reported incidence of graft failure is definitely low: 1/59 in the EBMT survey,[50] 1/41 in the MD Anderson series,[51] 1/26 in the Canadian experience.[52] The few rejections occurred in transplants with 1-2 antigen disparity. Platelet engraftment is also prompt, with median time to achieve an absolute platelet count (APC) of 20×10^9/L ranging between 10 and 18 days in the reported series.

Platelet more than neutrophil engraftment may be affected by acute GVHD or CsA toxicity as well as by early relapse or progressive disease.[50] In a recent report by the Genoa group a second infusion of PBSC without conditioning was required to achieve full engraftment of platelets in three out of thirty-one patients.[40]

The prompt engraftment offered by PBSC implies a reduction in transfusion need. The reported transfusion requirements range from 2 to 10 packed red cell units and from 3 to 12 platelet units.

Furthermore, GVHD prophylaxis may adversely influence the time to engraftment. In particular, methotrexate given for GVHD prophylaxis delays neutrophil and platelet engraftment.[53,54]

Growth factors, mainly G-CSF, have been employed in several studies to speed-up engraftment. Urbano-Ispizua reported that G-CSF given to patients not receiving methotrexate accelerates neutrophil recovery (p=0.001); median time to $> 20\times10^9$/L platelets was significantly delayed (p 0=0.01), although the time to reach 50×10^9/L platelets was not affected. No difference in engraftment kinetics was seen between cryopreserved and fresh PBSC when G-CSF was administered following transplantation.[50,55]

The studies carried out so far are not sufficient to draw definitive conclusions about engraftment with PBSC as compared to engraftment with BM. Randomized studies are still in progress and results are not yet available. Most information comes from comparisons of PBSC results with historical data from BM transplants.

A highly informative study was reported by the Seattle group, which compared 37 PBSC transplanted patients with a historical group of 37 bone marrow recipients.[56] Patients were well matched for diagnosis, disease stage, age and graft versus host prophylaxis. Faster neutrophil engraftment, 14 versus 16 days to reach more than 0.5×10^9/L (p=0.0063), and earlier achievement of platelet transfusion independence, 11 versus 15 days (p=0.0014), were observed in PBSC recipients compared to the BM control group. Consequently, the median number of platelet units transfused was 24 versus 118 (p=0.0001) and the median number of red blood cell units transfused was 8 versus 17 (p=0.0005) in the PBSC group and in the BM group, respectively.

Similar results have been reported by Russel *et al.*:[52] duration of aplasia for both neutrophils (p=0.0002) and platelets (p=0.0003) was significantly reduced in patients receiving PBSC compared to BM recipients. Interestingly, the advantage of PBSC was also maintained if methotrexate was used as GVHD prophylaxis.

A recent report by Rosenfeld *et al*.[57] evaluated 19 patients transplanted with PBSC. No growth-factor was employed in the post transplant phase. Significantly faster neutrophil recovery was observed in PBSC transplanted patients compared to historical control group transplanted with BM (p=0.01). However, the difference was not significant when the PBSC group was compared to BM recipients given G-CSF in the post-transplant phase.

More recently, a prospective non-randomized study was carried out by the M.D. Anderson group.[39] The study included 74 adults transplanted with HLA-matched related donors. Thiotepa,

busulfan and cyclophosphamide were employed as preparative regimen. The patients were divided into 3 cohorts: Group 1 received BMT using CsA and MTX as GVHD prophylaxis, Group 2 received marrow using CsA and MP, and Group 3 received PBSC with CsA and MP. All patients were given G-CSF post-transplant. Median time to neutrophils > 0.5×10^9/L was 17, 9 and 10 days, and to platelets > 20×10^9/L was 32, 25 and 18 days in Groups 1, 2 and 3, respectively. The use of CsA and MP for GVHD prophylaxis, rather than the source of engrafted cells was shown to be the most important factor for rapid neutrophil and platelet recovery. Provided that CsA/MP was used for GVHD prophylaxis, platelet transfusion requirement was found to be significantly lower in PBSC than in BMT recipients (p=0.04). Significant differences concerning regimen-related toxicity were seen for grade 2-4 stomatitis only between the BMT group using MTX in GVHD prophylaxis and the PBSC group using MP.

Correlation between engraftment kinetics and quantity of PB cells infused is still an open question. The absolute number of nucleated PB cells or $CD34^+$ cells did not correlate with time to neutrophils > 0.5×10^9/L or with time to platelets > 20, > 50 or > 100×10^9/L in a study by Rosenfeld *et al*.[57] Similarly, Urbano-Ispizua *et al.* did not find any correlation using several cut-off values of $CD34^+$ cells at 2.5, 3, 4, 5.5 and 7×10^6/kg. In contrast, Roy *et al*.[58] reported a correlation between $CD34^+$ cells infused and engraftment using a mobilization regimen with G-CSF at a dose of 5 µg/kg. In a large series published by the M.D. Anderson group,[37] in univariate analysis of patients not given MTX prophylaxis the number of total nucleated cells infused positively affected ANC recovery. Moreover, platelet recovery was positively influenced by the number of $CD34^+$ cells, as well as by young age and sex mismatching.

Immune reconstitution after transplantation of peripheral blood stem cells

Patients undergoing allogeneic BMT experience a prolonged period of profound cellular and humoral immunodeficiency, mainly due to complete pre-transplant destruction of the host lymphohemopoietic system, the use of immunosuppressive drugs for GVHD prophylaxis and the development of GVHD.[59-61] This immunodeficiency lasts until stem cells and mature lymphocytes contained in the transplanted marrow repopulate and reconstruct the hematopoietic and lymphopoietic systems which had been destroyed by the pre-transplant conditioning regimen. In particular, immunological reconstitution after BMT is considered to be dependent on two distinct phenomena.[59,60] In the early post-transplant period, there is an expansion of mature donor-derived lymphocytes transferred with the graft, a process influenced by both the recipient's environment and the *cytokine storm*[62] related to the transplant procedure. Thereafter, naive lymphocytes derived from the differentiation of donor hematopoietic stem cells colonize the lymphoid organs of the recipient and sustain the late immune response.

The crucial role of the first step in immunological recovery is demonstrated by the observations that patients receiving a T-cell depleted transplant are at particular risk for infections and that patients transplanted using donors either recently vaccinated against or immune to a certain pathogen usually have a more rapid recovery of specific T-cell response than ones who received bone marrow from unprimed donors.[63-65] Formal proof of the contribution of transferred donor-derived lymphocytes to recipient immune reconstitution has been recently reported.[62] In fact, using the combination of a cell culture method and a PCR amplified technique to study tetanus toxoid (TT)-specific T-cells clones, it was possible to demonstrate that patients after BMT display a small response that can be accounted for by a few donor-derived clones and that the T-cell clones transferred with the transplant were still detectable within the donor polyclonal T-cell lines for up to at least 5 years after BMT. Moreover, the vaccination of donors with TT before BMT resulted in a more relevant transfer of antigen-experienced T-cells.[66]

The expansion of mature donor-derived lymphocytes transferred with the graft in recipients of peripheral blood stem cell (PBSC) transplantation could be expected to be more efficient than patients given BMT, in view of the higher number of donor lymphocytes transferred. However, at present, few reports specifically addressing the question of immune recovery after transplantation of PBSC are available.

Ottinger *et al*.[67] demonstrated that, compared to BMT recipients, patients who were given a PBSC transplant had a more rapid recovery of both naive and memory $CD4^+$ cells (expressing the RA and RO isoforms of the CD45 molecule, respectively) whose counts significantly exceeded those observed following marrow transplantation. This determined that in patients receiving PBSC transplantation the characteristic inversion of the $CD4^+/CD8^+$ ratio observed after BMT was not encountered. Furthermore, the B-cell levels and, at least for the first 2 months after transplantation, the monocyte counts were augmented. Since monocytes of granulocyte colony-stimulating factor (G-CSF)-mobilized donors have been demonstrated to reduce the responsiveness of alloantigen specific T-cells, the increase in their count could contribute to the low incidence and reduced severity of acute GVHD reported after transplantation of PBSC.[68] Moreover, it must be noted that there is a prompt recovery of the lym-

phocyte counts after transplant of PBSC coupled with an enhanced *in vitro* response of lymphocytes to aspecific polyclonal activators (phytohemagglutinin and pokeweed mitogen) and to recall antigens (TT, *Candida*). The most likely hypothesis for explaining this accelerated recovery of helper T cells, B lymphocytes and monocytes is that the number of lymphocytes infused for each subset is more that one magnitude higher in recipients of PBSC transplant than in patients given BMT. However, alternative mechanisms cannot be excluded.

Similar results in terms of more rapid recovery of $CD4^+$ cells have also been reported by Bacigalupo *et al.*[40] in adults with advanced leukemia who received high-dose chemotherapy followed by G-CSF mobilized PBSC. More recently, two additional reports have further confirmed that recipients of PBSC transplants have a faster recovery of both naive and memory helper T cells.[69,70] Moreover, one of these studies documented that patients experiencing a more rapid recovery of the lymphocyte count had a significantly better probability of survival after transplantation.[69]

Whether the improved immune reconstitution observed after transplantation of PBSC is associated with a lower incidence of infectious complications still remains to be documented. In one of the previously mentioned studies,[69] the actuarial risk of reactivation of human cytomegalovirus (HCMV) infection in patients given a PBSC transplant was comparable to that observed in a historical control group of BMT recipients. This could be attributed to a greater viral load infused with the graft and correlated with the very large number of nucleated cells that can harbor HCMV transfused. Nonetheless, since the use of donor-derived adoptive immune therapy has been shown to be able to cure or prevent HCMV-related interstitial pneumonia and EBV-induced lymphoproliferative disorders,[71-73] it can be hypothesized that patients given PBSC transplants, with a more efficient transfer of antigen-experienced lymphocytes, may have a reduced incidence and/or reduced severity of infectious complications. Support for this theory is provided by the study reported by Bensinger *et al.*[74] in which a lower number of deaths from infectious complications was observed in patients given PBSC as compared to a historical group of BMT recipients.

Acute and chronic GVHD

PBSC collections contain a large number of T-cells – approximately 10 times more than unmanipulated marrow grafts.[75] Therefore concern for increased incidence and severity of GVHD after their infusion into an allogeneic host has been and still is a major issue after PBSC transplantation. Here we analyze the results reported so far in the most recent peer-reviewed studies published. Because of the relatively short follow-up of these studies, the assessment of chronic GVHD (cGVHD) is less complete and less accurate than that of the acute form. Some of the studies in fact do not address the problem of cGVHD. Acute GVHD, on the other hand, can now be evaluated in a rather significant number of patients. We shall look first at the characteristics of the studies, then analyze

Table 2. Main characteristics of the studies reviewed.

Ref	Type Of study	Period of study	Median Follow-up days (range)	N° pts	Median age yrs (range)	2nd transplants	Hla family mismatches	Phase of the disease
74	SC	12/93-11/95	nr	37	38 (20-52)	NO	NO	Advanced 100%
52	MC	5/93-6/95	nr	26	40 (1-54)	3 (11%)	6 (23%)	High risk 23 (88%) Standard risk 3 (12%)
40	SC	nr	136 (6-228)	31	44 (19-55)	NO	3 (10%)	Advanced 28 (90%) Early 3 (10%)
55	SC	nr	270 (180-600)	25	43 (17-57)	1 (4%)	NO	Relapse 21 (84%) Remission 4 (16%)
76	MC	3/94-7/96	nr	24*	37 (16-57)	1 (4%)	1 (4%)	Early 10 (42%) Advanced 14 (58%)
54	SC	1/94-4/95	nr	33	36 (12-53)	8 (24%)	NO	Early 12 (36%) Advanced 21 (64%)
39	SC	32 months	nr	19	Not detailed	1 (5%)	NO	Early 18% Advanced 82%
77	SC	3/94-4/95	111 (15-402)	17	33 (16-52)	NO	NO	Early 6 (35%) Advanced 11 (65%)
50	MC	1994	nr	51°	39 (2-54)	NO	NO	Early 15 (25%) Advanced 44 (75%)

*LEGEND: SC = single center; MC = multicenter; n.r. = not reported; *includes 1 pt with SAA; °includes patients from studies #40, 76 and 54.*

Table 3. Transplant modalities, aGVHD and cGVHD.

Ref	Fresh or cryo-preserved cells	Conditioning regimen* N° pts	G-CSF post TX	GVHD prophylaxis N° pts	Acute GVHD				Chronic GVHD		
					n. evaluable	grade II-IV	grade III-IV	GVHD related/ total deaths	n. evaluable	limited	extensive
74	F	TBI 32 Bus 5	no	CsA/MTX 19 CsA/PDN° 18	35	13 (37%)	5 (14%)	1/15	17	3 (18%)	4 (24%)
52	C	TBI 18	no	CsA/MTX 26	nr	37%	nr	nr	nr	53% overall	
40	F	Thio-CTX 31@	no	CsA/MTX 31	31	17 (55%)	4 (13%)	4/12	28	15 (53%)	7 (25%)
55	C	Bus 25	yes	CsA/PDN 25	25	11 (42%)	6 (22%)	3/7	nr	nr	nr
76	F^	Bus 22 Other 3	no	Csa/MTX 23 CsA 1	22	10 (45%)	2 (9%)	0/7	16	1 (5%)	9 (50%)
54	F 21 C 1	TBI 17 Bus 16	yes (11 pts)	CsA 2 CsA/MTX 22 CsA/PDN 9	32	11 (34%)	7 (22%)	nr	11	4 (36% overall)	
39	C	Bus 19	yes	FK-506/PDN 19	nr	22%	11%	nr	nr	nr	nr
77	F	Bus 17	no	CsA/MTX 17	10	3 (33%)	4 (24%)	4/4	10	3 (33%)	0
50	F 49 C 10	TBI 22 Bus 22 Thiotepa 11	yes (14 pts)	CsA/PDN 7 CsA 6 Other 9 Other 4	57	30 (50%)	14 (23%)	7/29	49	17 (35%)	13 (26%)

**Conditioning regimen mainly based on; @Regimen not including TBI or busulphan; CTX = cyclophosphamide; ^cells infused over 2 days: apheresis of day 1 stored at 4°C until infusion; °PDN=prednisone; n.r.=not reported*

acute and cGVHD separately and finally make comparisons between marrow and PB blood transplants. A set of tentative comments will be made at the end of the chapter.

Type of studies. Selected studies of PBSC transplantation for hematological malignancies are reported in Tables 2 and 3. Several of them have been analyzed in the section on hematological recovery. Table 2 focuses on the main demographic characteristics, while Table 3 gives details of the transplant procedure and results of acute and chronic GVHD where applicable. Six studies are from a single institution,[39,40,55,75-77] while three are from several centers;[50,52,54] one study from the EBMT Group,[50] multicentric in nature, also reports several patients included in four of the other studies – a typical example of double reporting – so that its results reinforce what has already been observed. The figures from this last study were not calculated in any further statistical analysis in order to avoid the error of counting some of the patients twice. However, they are useful for comparisons and have been left in the tables. The total number of patients is 212. None of the studies is prospective or randomized, but four[52,55,75,77] compare the results of PBSC with those of marrow, although using different methods. We shall have to wait some time before seeing the results of the two prospective randomized studies comparing marrow and PBSC transplantation which are now in progress in Europe and the US; for the moment, the reports analyzed here represent the best we have. The 8 studies took place recently, between late 1993 and 1995, and mostly dealt with adults (median age 38 yrs, with a range from 1-57), but some included pediatric patients. Transplants were from fully HLA-identical siblings in 96% of the cases, but a minority received cells from family donors mismatched for one HLA antigen; a minority of patients (5 to 10%) also received a second allo transplant, usually from the original sibling who had donated the marrow. Patients showed a typical spectrum of hematological malignancies for which transplant is indicated. The majority (median 83%) were in advanced phases of their diseases, although definitions are quite variable with the term *high-risk* being used as a synonym for advanced phase, but 17% had early phase or low-risk disease at the time of transplant. These proportions differed widely within studies, some including 100% advanced diseases and others only 60%, with many more early phase patients. Pretransplant regimens were obviously different, but despite their apparent disparities they can be grouped into those based on busulphan (54%) or

TBI (31%). Only two studies differ considerably from the rest of the series: the Genoa group[40] purposely employed a low intensity regimen based on thiotepa and cyclophosphamide to reduce toxicity in a rather old patient population. The MD Anderson Hospital,[39,55] on the other hand, used a very intensive regimen combining busulphan, thiotepa and cyclophosphamide in a population of similar age. Another difference is represented by the processing of the collected PBSC: in 130 cases (61%) they were infused fresh and in 82 cases (39%) they were cryopreserved instead until infusion. Finally, GVHD prophylaxis was not uniform: it was based on a combination of CsA and short-course methotrexate in 130 (62%) of the cases, and on CsA plus prednisone in 42 cases (20%); only one study[38] reports 19 patients (9%) who received a combination of tacrolimus and prednisone. CsA alone was used in three patients (1.4%).

Acute GVHD. The incidence of aGVHD, grade II to IV, was about 40% on average. The Genoa study[40] reported a 55% incidence, but it also included the oldest patients in the series; the lowest incidence, 22%, was reported in the series from the MD Anderson Hospital where tacrolimus was used for GVHD prophylaxis.[39] The incidence of severe aGHVD, i.e. grade III and IV, was on average 16% (range 11-24%). An interesting point is the fact that while most studies showed a direct correlation between the overall incidence of GVHD and severe GVHD – the latter being about half of the former – others did not. Two studies from Italy[40,76] which reported an overall incidence of over 50% also showed a low incidence of severe GVHD, which means that grade II accounted for the majority of the cases. No correlation could be made from the existing data between the variables known to influence aGVHD[78] and the results either within the single studies as discussed by their authors or by combining data as in this review. Of interest, on the specific issue of PBSC, no correlation was found with the number of T-lymphocytes infused or with the use of fresh or cryopreserved cells. However, it should be noted that the highest incidences of severe aGVHD (22-24%) were reported when prophylaxis was based on CsA/prednisone,[55] which is perhaps less effective than CsA/MTX or in the Spanish study which reported data from multiple institutions[54] with a good proportion of patients receiving CsA/prednisone for GVHD prophylaxis. Nevertheless, a high incidence was also reported in a study from Brazil where CsA/MTX was used in all cases.[77] Mortality from aGVHD is not reported in all studies, as shown in Table 3; those giving the causes of death often do not mention, when infection was the main cause, whether GHVD was associated. However, considering the causes of death of 47 events analyzable in detail, GVHD was the main cause in 12 (25%). Incidentally, this figure is higher than the overall incidence of grade III-IV GVHD, but data on death are reported for fewer patients than data on GVHD.

Chronic GVHD. The number of patients analyzable for cGHVD is smaller than for aGVHD; survival > 90, 100 or 150 days is the requisite for evaluation. In addition, some studies give many details on cGVHD while others do not address the issue[55] or mention it very briefly.[39,52,54] We calculate that slightly more than 110 patients are evaluable. The overall incidence ranged from 36% to 78%; considering the four studies which give detailed information, the extensive form of the disease occurred with nearly the same frequency or less than the limited form in three studies,[40,56,77] while one series reported a striking incidence of the extensive form, with the limited one being only minimally represented.[76] In that study cGVHD developed *de novo* in 5 out of 10 patients, at variance with the low incidence of aGVHD observed earlier. Another interesting observation is contained in a study from Seattle:[56] of 10 patients at risk, who had received CsA/prednisone for prophylaxis, 6 developed cGVHD, while only 1 of 7 given CsA/MTX did so.

Comparisons between marrow and PBSC transplantation. Four studies[52,55,56,77] compared the incidence of GVHD after PBSC or marrow transplantation. These studies were carried out using matched-pair analysis with a historical control group of marrow recipients who were matched for diagnosis, disease and disease phase at transplant, age, GVHD prophylaxis[56] or age and disease status.[52] One study does not give the characteristics of the marrow recipients.[77] The Seattle study found a lower incidence of grade II-IV aGVHD for PBSC recipients – 37% vs 56% – severe GVHD (grade III-IV) was even more impressively lower in PBSC recipients, 14% vs 33% of marrow transplants. However, due to the small number of patients these data are not statistically significant. The overall incidence of cGVHD was similar in the two groups, with a tendency toward more severity in the PBSC than in the marrow group (42% vs 26% for any grade of clinical cGVHD), but again this was not statistically significant. The striking effect of MTX in the GVHD prophylaxis regimen observed in this study has already been mentioned. The multicenter Canadian study[52] reported a higher incidence of both aGVHD and cGVHD for the PBSC group than for the marrow recipients: 37% vs 21% grades II-IV aGVHD and 53% vs 48% for cGVHD, respectively. A different kind of comparison can be made in a study from the MD Anderson Hospital,[55] where patients with advanced hematological malignancies received, over a 3-year period, the same conditioning protocol but different forms of GVHD prophylaxis and different sources of allogeneic stem cells. The PBSC patients could be compared to the marrow group who received CsA/prednisone as GVHD prophylaxis: severe aGVHD was slightly less in the

PBSC group, 22% vs 33%, but this difference was not statistically significant. No data on cGHVD were provided. The Brazilian study[77] reported more aGVHD in the PBSC than in the marrow group, grade III-IV 4/17 vs 3/21, but again this was not statistically significant. No comparative data on cGVHD were given.

Comments. The fear of an unacceptably high rate of severe and perhaps uncontrollable acute GVHD after allo PBSC can now be allayed with a certain degree of confidence on the basis of the data analyzed here. In a population of adults with mainly advanced hematological malignancies who sometimes received second transplants or not fully HLA-identical grafts and, most important, were often not given the best GVHD prophylaxis available, acute GVHD was no more than what is expected with marrow transplants. Similar conclusions had been reached in an earlier review on the subject,[56] although on a much smaller number of patients. It is more difficult to say whether GVHD is slightly more severe than after conventional marrow grafting, considering the wide variation in its occurrence, due to the multitude of factors which influence it. A broad comparison of the published data indicate that GVHD observed after PBSC is higher than in the best marrow series[79] but not worse than what is described in the large reports from registries.[80] Four studies have attempted a comparison with retrospective marrow transplants and none found significantly increased aGVHD incidence or severity. It is interesting to speculate why after infusion of 1 log more T-lymphocytes as compared to marrow aGVHD is not increased. One explanation is that any number of T-cells, once the 10^5/kg threshold has been surpassed,[81] is already high enough to cause GVHD and even a 10-fold increase, with respect to the marrow, does not make any difference. Perhaps an extraordinarily large number of T-cells infused, that is usually not reached after G-CSF mobilization, for example 3 or 4 logs, could be the next threshold above which more severe GVHD would regularly occur. Data from a study in Seattle, where 1500×10^6/kg donor buffy-coat mononuclear cells were intentionally infused soon after BMT in patients with advanced disease to enhance a graft-versus-leukemia effect did show that acute, severe GVHD was indeed increased; in that study, unfortunately, GVHD prevention was based on MTX only so comparisons with today's practices are not possible.[82] Other explanations for why aGVHD does not increase relate to the possible biological modifications of lymphokine production induced by G-CSF. For example, in mice G-CSF has been demonstrated to induce a polarization of T-lymphocytes towards the production of type-2 cytokines (namely IL-4 and IL-10), which display an anti-inflammatory effect. Such polarization was shown to be long-lasting and was associated with a significant reduction in the severity of GVHD after transplantation of these cells into allogeneic mice recipients.[83] These results do not seem to be attributable to a direct effect of G-CSF on T-cells, since this subset of lymphocytes rarely expresses G-CSF receptors. Instead, these findings could be explained by the anti-inflammatory effects of G-CSF; in fact, administration of G-CSF decreases tumor necrosis factor (TNF) secretion.[84] Moreover, in normal subjects G-CSF is able to increase the production of two important cytokine antagonists such as soluble TNF-receptor and IL-1 receptor antagonists.[85]

Finally, leukapheresis products from G-CSF-mobilized donors contain a large number of monocytes. These cells have been demonstrated to significantly reduce the alloantigen specific proliferative response of T-lymphocytes.[67] Also, monocytes from subjects treated with G-CSF or GM-CSF can induce the apoptosis of T-lymphocytes via the interaction of the FAS molecule with its ligand.[86] The use of G-CSF may inhibit the function of monocytes as antigen presenting cells and this, in turn, may explain the ability of this cytokine to polarize T-cells towards an anti-inflammatory cytokine profile. These findings could also contribute to explaining the unexpectedly low incidence and reduced severity of GVHD after transplantation of PBSC. However, more studies on the characterization of G-CSF mobilized lymphocytes are needed.

With regard to cGHVD, it appears from this analysis that there is a trend toward a slightly increased incidence after PBSC, although not all individual studies had the same results. The clinical presentation of cGVHD was reported as peculiar in two studies, with many *de novo* cases[31,75] but also with a high response to treatment.[49] It should be noted that early data from the M.D. Anderson on PBSC transplants also reported an increase in cGVHD, with more liver and gastrointestinal manifestations compared to the marrow.[87] At the time of this writing, several patients were still on immunosuppressive treatment so the full magnitude of cGVHD will be appreciated only in the future after withdrawal of CsA, which is a critical time for the development of the syndrome. Furthermore, in a study on aplastic anemia[88] the infusion of donor buffy coat cells was associated with a significant increase of cGHVD. However, this increase of cGVHD was not observed in malignancies in the study.[82] Clearly a longer follow-up of a much larger number of patients is needed to answer the question of cGVHD.

Transplantation of enriched allogeneic CD34+ cells

As reported above, allogeneic PBSC transplantation results in the infusion of approximately 1 log more T-cells than conventional BM transplantation. Thus, in order to reduce the potential risk of severe aGVHD, several investigators have attempted to

remove T-lymphocytes from allogeneic grafts. The only technique that has been utilized so far for T-cell depletion has been the positive selection of hematopoietic CD34+ cells.

The CD34 antigen is present on the earliest identifiable progenitor cells and committed myeloid precursors, whereas it is not expressed on mature myeloid and B- and T-lymphoid cells.[89] However, CD34+ cells co-expressing both T-lymphoid (CD2, CD3, CD7) and B-lymphoid markers (CD19) are likely to be the early precursors of the T- and B-lymphoid lineages.[90] Preclinical studies have also shown the capacity of positive selection of CD34+ cells to eliminate 3 to 4 logs of T-cells, coupled with a substantial recovery of hematopoietic progenitors.[91,92] More recently, transplantation of autologous CD34+ cells has been proven to reconstitute normal hematopoiesis in cancer patients treated with myeloablative regimens.[93-96]

Based on these premises, Link *et al.*[97] transplanted 5 patients with unmodified marrow and CD34+ selected PBSC and 5 patients with enriched marrow and PB CD34+ cells. They concluded that hematopoietic recovery was accelerated with respect to marrow allografts without an apparent increase in aGVHD following conventional CsA and MTX prophylaxis. In a subsequent study,[98] the same authors transplanted 10 individuals with positively selected circulating CD34+ cells alone. The patients were grouped according two different regimens of aGVHD prophylaxis: CsA alone or CsA and MTX. The median grades of aGVHD were 3 in group I (CsA) and 1 in group II (CsA plus MTX). Two patients in group I died from aGVHD and 2 leukemic relapses occurred in group II. Complete and stable donor hematopoiesis was shown in all patients with a median follow-up of 370 days (range 45-481). It was concluded that despite a 3-log reduction of T-cells by CD34+ cell enrichment, CsA alone was not sufficient to avoid severe aGVHD.

More recently, Bensinger *et al.*[99] transplanted 16 patients with advanced hematologic malignancies with HLA-identical highly enriched PB CD34+ cells. Prophylaxis against aGVHD was CsA alone for 5 patients and CsA plus MTX for 11. A median of 8.96×10^6 CD34+ cells/kg of patient body weight were infused with a median purity of 62%. Positive selection of stem cells resulted in a median 2.8-log reduction of T-cells. Despite the prompt and sustained engraftment, 8 out of 16 patients died between 3 and 97 days post-transplant of transplant-related causes and 1 of progressive disease. Grade 2-4 aGVHD occurred in 86% of patients and 6 out of 8 evaluable patients developed clinical chronic GVHD.

More promising results have been reported by Urbano-Ispizua *et al.* (1997), who recently transplanted 20 acute and chronic leukemia patients with allogeneic CD34+ cells. The median number of CD34+ cells and CD3+ cells infused was 2.9×10^6/kg and 0.42×10^6/kg, respectively. The patients were conditioned with fractionated TBI (total dose 13 Gy in 4 fractions) and cyclophosphamide 120 mg/kg. Additional GVHD prophylaxis included CsA and methylprednisolone. No patients developed grade II-IV aGVHD. The overall procedure was associated with low morbidity and no transplant-related deaths occurred within the first 100 days. Although the median follow-up (7.5 months) is rather short for a full evaluation of cGVHD incidence and disease relapse, the absence of extensive cGVHD and the low rate of disease recurrence (only 3 out of 20 patients relapsed) encourage further studies in this direction. In comparison with previous studies,[99] it should be noted that the median age of the patient population was 40 years and only 35% of the individuals were older than 45 years. Moreover, the majority of the leukemic patients were transplanted in the early phase of their disease. Both these parameters are generally associated with lower transplant-related mortality and a lower incidence of severe GVHD.

Although further studies involving larger numbers of patients are currently in progress, these results, taken together, demonstrate that infusion of CD34+ selected PBSC results in rapid and stable engraftment. However, transplantation of purified stem cells may induce a higher rate of acute and chronic GVHD than expected, thus requiring full GVHD prophylaxis. Therefore this approach for T-cell depletion should be carefully evaluated in the setting of HLA-identical PBSC transplantation and weighed against the potential increased risk of disease relapse, and perhaps delayed immunological reconstitution, and the increased cost of the procedure.

Allogeneic PBSC from haploidentical familial donors: the mega-stem-cell dose concept

Allogeneic BMT has been largely confined to patients who are HLA-identical to their donors. At present, only about 30-35% of patients who might benefit from allogeneic BMT have an HLA-identical sibling. The establishment of large registries of HLA-typed individuals during recent years has led to a substantial increase in transplants from unrelated donors.[100-102] Although 40 to 50% of patients are successful in locating HLA-A, B, DR-matched unrelated donors, many patients still fail to find an appropriate donor.[103,104] In contrast, nearly all patients have an HLA-haploidentical relative (parent, child, sibling,) who could serve as a donor.

The feasibility and safety of transplants from partially matched family members have been investigated and the results of these studies have demonstrated that HLA matching is a critical and limiting factor in marrow transplantation.[105-107] In published

works on mismatched transplants, there has been no large study involving patients mismatched with their donors by one full haplotype. These experiences have been limited because the problems of transplant increase with the number of antigenic disparities between donor and host.[106] In 2- or 3-antigen mismatched transplants, studies by the Seattle program[105] and the *International Bone Marrow Transplant Registry*[108] reported graft failure in 20 to 30% of cases. The reported incidence of acute GvHD (grade II or greater) varied from 34% to 100% overall, but in 2- and 3-antigen mismatched patients the incidence was at least 80%.[105,106] Severe GvHD was a greater problem than graft rejection, preventing more widespread use of mismatched related transplants during the latter 1970s and 1980s.

By contrast, extensive experience in severe combined immunodeficiency (SCID) patients has shown that GVHD is largely preventable, even in 3-antigen mismatched transplants, when a 3-log T-cell depletion of the donor bone marrow is achieved.[109,110] In 1981 Reisner *et al.* reported the first case of leukemia treated with a T-cell depleted marrow transplant from a haploidentical, 3-loci incompatible, parental donor.[111] There was full engraftment and no GVHD. Subsequently, clinical trials in mismatched-sibling BMT for patients with leukemia were begun using the lectin or other T-cell depleting methods which included monoclonal antibodies with complement or conjugated to toxins, and counterflow centrifugal elutriation (reviewed in ref. #112). It was determined that the threshold dose below which GVHD was not seen in matched patients was 2.0×10^5 T cells/kg.[113] However, early enthusiasm for all methods was soon tempered by an increased incidence (> 50%) of graft rejection.[114]

In exploring the problem of failure in mismatched grafts, the inadequacy of immunosuppression was documented by the observation of residual host lymphocytes in patients who failed to engraft after being conditioned with conventional preparative regimens and given T-cell depleted mismatched transplants.[114] Work in esperimental models has shown that incompatible T-cell depleted transplants can be successfully performed by manipulating the conditioning regimen and/or the graft composition.[115] The immunologic response of the remaining host immune system against the graft can be overcome by increasing the total dose of TBI[116] or by adding selective anti-T measures with minimal toxicity, such as splenic irradiation[117] or *in vivo* treatment with anti-T monoclonal antibodies.[118] Engraftment is also improved by increasing the myeloablative effect of the conditioning regimen through the use of dimethyl-myleran, busulfan or thiotepa, given with TBI.[119,120]

Different cytoreductive agents or radiation regimens were therefore added to the basic conditioning protocols used for conventional BMT. Although a marked beneficial effect was found in recipients of T-cell depleted HLA-identical bone marrow upon adding ATG and thiotepa to TBI and cyclophosphamide,[121] none of these agents were found to be useful in recipients of T-cell depleted haploidentical 3-antigen incompatible transplants. Others, using the monoclonal antibody Campath-1G instead of ATG, have observed similarly disappointing rejection rates.[122]

Concerning the composition of the graft, Lapidot *et al.* showed that megadoses of T-cell depleted incompatible bone marrow inoculum could obtain full donor-type engraftment in mice treated with sublethal irradiation, or presensitized with donor lymphocytes or partially reconstituted before the transplant by adding back a controlled number of host-type mature thymocytes.[123]

The means of overcoming graft failure elucidated in the experimental model can be applied in the clinical setting by combining approaches that increase both the conditioning of the host and the size of the stem cell inoculum. The major advance that finally made full haplotype-mismatched transplantation possible in leukemia patients was the availability of rhG-CSF[124] and the experience in autologous transplants in which G-CSF was used to mobilize high numbers of stem cells into the blood of patients without significant side effects.[125] Their employment made it feasible to increase the number of donor stem cells to a level which, in animal models, made transplantation across the histocompatibility barrier possible.[117] On the basis of these concepts, the BMT team at the University of Perugia first introduced the megadose cell transplant in full haplotype-mismatched leukemia patients.[126] After a conditioning regimen which included 8 Gy TBI in a single fraction at a fast dose rate (16 cGy/m), thiotepa (10 mg/kg), rabbit ATG (20 mg/kg in 4 days) and cyclophosphamide (100 mg/kg in 2 days), advanced leukemia patients, mostly adults, were given the combination of marrow and G-CSF-mobilized blood stem cells. Donors compatible with the patients for only one haplotype and 3-antigen disparate on the other haplotype underwent bone marrow harvest followed within a few days by treatment with G-CSF (12 μg/kg/d × 7 days). Four leukaphereses of progenitor cells were performed starting on the fourth day. The marrow as well as the leukapheresis product were each depleted of T-cells using soybean lectin agglutination and E-rosetting.[127] Both the $CD34^+$ cells and CFU-GM were increased 7- to 10-fold over bone marrow alone, and the average number of $CD3^+$ cells infused was 2.2×10^5/kg recipient body weight. Following conditioning and stem cell infusion, patients received no additional GvHD prophylaxis. The results of the first 17 patients were reported in 1994[126,128] and subsequently 27 additional leukemia

patients, most of them in chemoresistant relapse at the time of transplant, were treated. For the first time a very rapid hematopoietic engraftment was observed in more than 80% of patients and, without any post-transplant prophylaxis, acute GVHD occurred in only 27%, and there was no significant chronic GvHD. As for survival, 7 patients are currently alive and disease free at a median follow-up of more than 3 years. The major complications observed in this pilot study were interstitial pneumonitis, which occurred in 43%, and infections in the setting of GvHD. Both were responsible for the 60% transplant-related mortality.

This pilot experience showed that the megadose cell strategy, together with a highly immunosuppressive and myeloablative conditioning, resulted in a high incidence of durable engraftment with significantly reduced GvHD comparable to historical experience with unmanipulated transplants. It also confirmed that in humans, as in mice, the stem cell dose plays a crucial role in overcoming HLA-histocompatibility barriers.[129] This concept is also supported by the recent work by Rachamin *et al.*[130] demonstrating that purified $CD34^+$ cells have a very powerful veto activity. They are able to specifically reduce, in a mixed lymphocyte culture, the frequency of CTL precursors against the stimulatory cells of the same subject and thereby help to overcome allogeneic rejection and enhance their own engraftment.

The approach to haplotype-mismatched transplants has evolved since Aversa *et al.*[126,128] originally proposed the megadose cell concept. With their initial protocol GvHD was decreased but not eliminated, and it contributed to transplant-related mortality, which was significantly greater than in matched patients receiving similar conditioning (Aversa *et al.*, unpublished data). These remaining problems were addressed in a subsequent trial, where it was possible to completely abrogate GVHD by improving the T-cell depletion method.[131] By processing the peripheral blood progenitor cells with an initial debulking of both mononuclear and T-cells with one-round E-rosetting followed by positive selection of $CD34^+$ cells with the Ceprate stem cell concentrator (CellPro Inc. Bothell, WA, USA), it was possible to infuse a median of 3×10^4 $CD3^+$ cells/kg and 13×10^6 $CD34^+$ cells/kg in 24 high-risk leukemia patients. Conditioning-related toxicity was also reduced by modifying pretransplant chemotherapy. As a substitute for cyclophosphamide, which was considered a possible factor in the early mortality in the first pilot study, fludarabine was tested. In fact, it had been shown to have powerful immunosuppressive effect in patients treated for lymphoproliferative disorders, even at doses which were not associated with significant extrahematologic toxicity.[132] Furthermore, in a mouse model TBI+fludarabine (40 mg/m^2/d $\times$ 5) was shown to provide an immunosuppressive effect comparable to TBI+cyclophosphamide.[133]

At present, a regimen including TBI in a single fraction, thiotepa, fludarabine and ATG followed by the infusion of T-depleted bone marrow plus T-depleted CD34-selected blood cells is being evaluated for toxicity and efficacy. The preliminary results of this study were recently presented at the American Society of Hematology meeting in Orlando.[134,135] As hoped, with the decrease in the number of T-cells infused and the modifications in conditioning, the problem of GvHD was largely prevented (only 2 patients developed grade II acute GvHD and one progressed to chronic GvHD); the engraftment rate was 95% and there was a decrease in transplant-related mortality to 29% compared to the previous 60%.

A more recent update on 48 patients was presented in Mannheim.[136] The abstract reports that 22/28 patients were in chemoresistant relapse at the time of transplant; age ranged from 4 to 53 years (median 27). Forty of 48 patients engrafted, grade II-IV acute GvHD occurred in only two patients and no one developed chronic GvHD. Twenty patients were alive and disease free at a median follow-up of 5 months (range 1-16). There were 11 relapses and 17 nonhematologic deaths. Transplant-related mortality was 35%.

An unsolved problem remains the slow immunologic recovery of engrafted patients that is responsible for infections. Counting of peripheral blood lymphocytes which exhibited a phenotype of NK cells ($CD56^+$), helper T cells ($CD3^+/CD4^+$), cytotoxic T-cells ($CD3^+/CD4^+$), cytotoxic T-cells ($CD3^+/CD8^+$) and B-cells (IgM^+) revealed early recovery (within 2-4 weeks) of NK cells and extremely delayed recovery of T cells. In particular, $CD4^+$ cells reached near normal values after 10 to 12 months.[137] In addition, the frequencies of T-cells responding to polyclonal activators in a sensitive limiting dilution assay were approximately 1 in 100 within the first post-grafting month and 1 in 10 at 10 months post-transplant (control responder cell frequencies are in the range of 1 in 2).[137] The low number of T-cells, combined with their functional peculiarities (i.e. failure to respond to TcR stimulation) are certainly implicated in the high frequency of infectious complications and are strongly indicative of a markedly distorted T-cell maturational process.

Interestingly, looking at post-transplant immune reconstitution, Albi *et al.* observed a large donor-type TcR-$\alpha\beta^+$ $CD8^+$ cell population that co-express NK-like receptors for specific MHC class I alleles.[137] NK cells expressed multiple, clonotypically distributed membrane receptors with different specificities for families of MHC class I alleles (termed *killer cell inhibitory receptors*, KIR). The interaction between these receptors and the appropriate alleles produces a signal which inhibits killing of the target

cells. Analysis of more than 900 clones revealed that 40% to 80% of these KIR+ T-cells exhibit NK-like functions, i.e. they were able to lyse class I-negative targets and were functionally blocked by the expression of specific class I alleles on target cells. Furthermore, these cells do not lyse autologous hemopoietic cells, but are able to lyse fresh leukemic cells.[137] This might suggest that they could provide a graft-versus-leukemia effect without causing GVHD.

In a period of twenty years transplants across the histocompatibility barrier have advanced from being experimentally to clinically possible. The principles outlined at the beginning – adequate cell dose, adequate immunosuppression and myeloablation, avoidance of GvHD – have been successfully combined. Two other groups have recently reported on successful engraftment in haplotype mismatched transplants by combining bone marrow and G-CSF-mobilized blood stem cells after CD34-positive selection for patients with advanced leukemia.[138,139] Refinements of this protocol should make haplotype mismatched transplants an attractive therapeutic option for patients with high-risk leukemia without a matched related or unrelated donor.

Furthermore, there are enormous potential applications of the concept of the stem cell dose for the future treatment of non-neoplatic diseases like aplastic anemia, Fanconi's anemia, SCID, thalassemia, and for induction of tolerance in organ transplantation. This approach should be applicable not only in mismatched transplants but also for overcoming problems which remain in the matched transplant setting, such as rejection in aplastic anemia, regimen-related toxicity in Fanconi's anemia and thalassemia.

Transplantation of allogeneic PBSC from unrelated donors

Two retrospective studies have recently suggested that the number of hematopoietic cells present in BM harvest correlates with the clinical outcome in the setting of stem cell transplantation from both HLA-identical siblings and from HLA-matched unrelated donors.[9,140] In the latter case, the number of cells infused has proven to be the most potent prognostic factor for survival. Therefore, given the much higher number of progenitor cells collected in primed PB as compared to conventional BM harvest, the use of PBSC appears to be a promising alternative for improving the results of transplantation from unrelated donors. In this regard, Ringden *et al.*[34] and Stockschlader *et al.*[141] recently reported their preliminary experience with transplantation of allogeneic PBSC from full-matched or 1-antigen mismatched unrelated donors. In particular, Ringden *et al.* transplanted 6 patients with high-risk hematologic disease. Four of them received allogeneic PBSC as primary treatment while 2 others were treated after a BM graft failure. Five PBSC collections were infused without any manipulation; in 1 case Campath-1 monoclonal antibody was used for T-cell depletion. In the German study, 1 AML patient in 2nd CR received purified CD34+ cells from an HLA-matched unrelated donor. The total number of patients transplanted is too small and the follow-up too short to draw any conclusion; however, these preliminary data showing a rapid rate of engraftment are encouraging, whereas the role of T-cell depletion remains to be clarified.

Transplantation of umbilical cord blood progenitor cells

The existence of hematopoietic progenitors circulating between the fetus and the placenta during gestation was first described in this Journal more than 20 years ago,[142] but their clinical application began only when it became evident that the progenitor cell content of CB was sufficient for bone marrow repopulation in pediatric patients given myeloablative chemo-radiotherapy.[143] In 1988, a patient affected with Fanconi's anemia was first transplanted with CB progenitor cells from his HLA-matched healthy sibling.[4] Subsequently, successful CB transplants (CBT) were sporadically reported in patients affected by both malignant and nonmalignant disorders.[5,144-147] The establishment of large CB banks in Europe and USA, and improvement of the methods of cell collection, manipulation and freezing have permitted a rapidly increasing use of CB progenitor cells, which are now extensively employed for allogeneic transplantation.[148-150]

The biological and functional characteristics of CB hematopoietic stem cells have been already reviewed by the Working Group.[12]

Clinical results after cord blood transplantation

As mentioned above, the use of human umbilical CB hematopoietic progenitors represents an alternative modality of transplantation. Advantages of CBT include ease of hematopoietic stem cell collection, absence of donor risks, low risk of viral contamination (*cytomegalovirus*, Epstein-Barr virus, etc.) and, for transplantation among unrelated individuals, prompt availability of hematopoietic stem cells. Over the past decade, placental blood has been used to transplant hundreds of patients (mainly children) and information on the rate and kinetics of engraftment and on the risk of severe acute or chronic GVHD is now available for CBT recipients from both related and unrelated donors.

In the two largest cohorts of patients transplanted from an HLA-identical sibling reported to date,[7,148] the probability of engraftment of donor hematopoiesis was 79% and 85%, respectively, even though it must be underlined that in the cohort

analyzed by Wagner and colleagues rejections were mainly observed in patients affected by bone marrow failure syndromes or hemoglobinopathies, which are diseases with a high risk of graft failure. In the cohort reported by Wagner *et al*., the median time to achieve granulocyte (PMN >0.5×10^9/L) and platelet (PLT >50×10^9/L) recovery was 22 and 49 days, respectively; these values were greater than those observed with BMT. Comparable time for PMN and PLT recovery were observed in the European experience.[7] In particular, in this latter report, patients receiving a higher number of nucleated cells (i.e. more than 37×10^6/kg) experienced faster engraftment than those given a lower number of cord blood progenitors, suggesting that the number of cells infused is the main factor influencing the rate of hematologic recovery. More prolonged periods of profound leukopenia and thrombocytopenia have also been described in children receiving CBT from unrelated donors. In fact, in the first two series of patients transplanted from an unrelated donor,[149,150] PMN recovery occurred in a median of 22 and 24 days, respectively, whereas the median time for PLT recovery was 82 and 67 days, respectively. The importance of the number of cells infused on the kinetics of PMN and PLT engraftment in the *Eurocord Transplant Group* experience was also observed in the group of patients given an unrelated CBT. Moreover, unlike BMT, where the use of hematopoietic growth factors has been demonstrated to hasten myeloid recovery significantly,[151,152] administration of these cytokines has produced conflicting results in CBT recipients. In fact, in the cohort of patients receiving CBT from HLA-identical or disparate family donors studied by Wagner *et al.*, the use of G-CSF or GM-CSF did not influence the kinetics of PMN reconstitution.[142] In contrast, in a group of children transplanted using unrelated CB units reported by the same authors, patients receiving hematopoietic growth factors experienced faster myeloid recovery than those who were not given the cytokines.[150]

The delayed rate of neutrophil engraftment and the conflicting data mentioned above could be explained by the infusion of fewer progenitor cells with CBT with respect to BMT, as suggested by the European experience, or, alternatively, by the particular characteristics of the proliferative, self-renewing and differentiating capacity of CB cells. A practical consequence of the above observation is that specific attention should be paid to the risk of infectious complications in children receiving CBT.

During the first few months after transplant CBT recipients show a steady, impressive increase in HbF whose values are significantly higher than those observed in patients receiving BMT. Moreover, the subsequent decline is usually less pronounced than that observed in normal children in the first year of life (Figure 1).[153,154] This preferential production of γ chains in erythroid progenitors seems to reproduce the normal ontogeny of erythropoiesis, even though the persistence of HbF levels higher than those observed in the first year of age suggests a more delayed switch from fetal to adult hemoglobin synthesis.

The dose of CB progenitor cells necessary to ensure early and sustained hematopoietic engraftment and favorable clinical outcome has still not

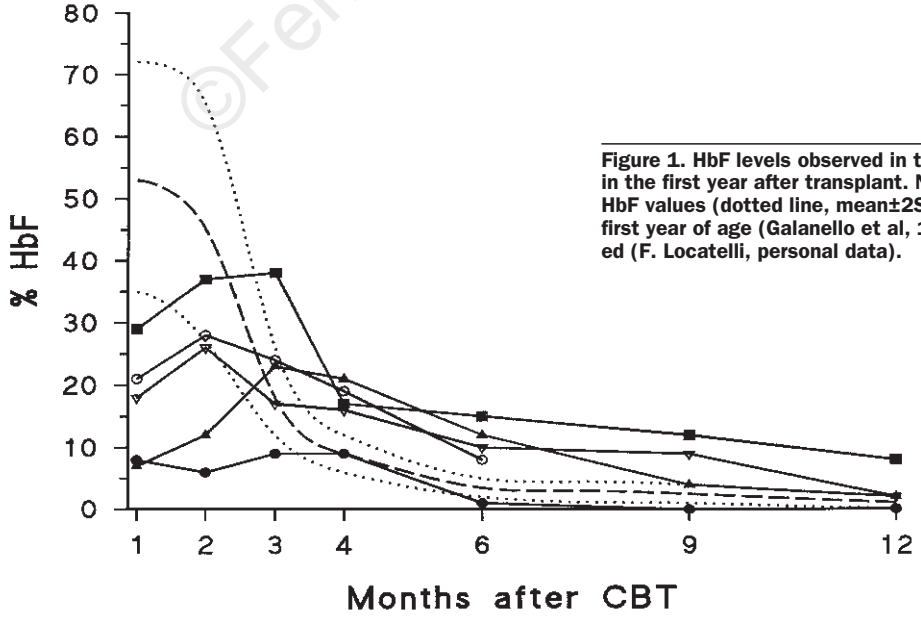

Figure 1. HbF levels observed in the 5 CBT recipients in the first year after transplant. Normal percentiles of HbF values (dotted line, mean±2SD) observed in the first year of age (Galanello et al, 1981) are also reported (F. Locatelli, personal data).

been precisely defined. Wagner *et al.*[148] claimed that the lowest dose of CB nucleated cells reported to be capable of yielding complete and sustained engraftment is 1×10^7/kg of recipient body weight. However, as previously mentioned, the *Eurocord Transplant Group* documented that a dose of nucleated cells available before thawing of fewer than 3.7×10^7/kg recipient body weight was highly predictive of both graft failure and poor survival after CBT.[7] The importance of this value also emerges from Kurtzberg *et al*'s experience.[149] Ten out of 13 patients undergoing CBT from an unrelated donor and having received fewer than 3.7×10^7/kg nucleated cells failed to benefit from the procedure. Although the importance of this cellular dose appears evident from these two reports, it should be noted that rarely is such a number of cells available in the case of adult patients. In fact, since the average leukocyte count in placental blood is about 10×10^6/mL and the average volume of donated blood is about 80 mL, the average number of nucleated cells before thawing in one cord blood unit may reach 800×10^6. About 30% of nucleated cells are lost during the thawing and washing procedure, and even though the loss mostly involves mature cells which have no role in transplantation, it is reasonable to expect fewer than 3.7×10^7/kg viable cells for patients with body weight greater than 30-40 kg.

The reduced immune reactivity of cord blood cells found a clinical counterpart in 38 children reported by Wagner *et al.*[148] who received CBT from an HLA-identical or 1-antigen mismatched sibling. In these patients, the incidence of grade II-IV acute GVHD and limited chronic GVHD was 3% and 6%, respectively, with no patient dying of GVHD. Confirmatory results were obtained by the *Eurocord Transplant Group,* which reported a 9% incidence of grade II-IV acute GVHD in CBT recipients from an HLA-identical relative. However, it is noteworthy that the same group documented a 50% incidence of grade II-IV acute GVHD in patients transplanted from an HLA nonidentical family donor.

In CBT performed between unrelated subjects with, in some cases, a disparity of 2-3 HLA antigens, the incidence of acute grade III-IV GVHD is reduced (approximately 10-20%)[7,149,150] with respect to that observed after unmanipulated BMT between unrelated subjects for whom, notwithstanding complete HLA identity between recipient and donor, the observed risk of acute grade III-IV GVHD reaches at least 30-40%. In particular, in the cohort of patients given CBT from an unrelated donor reported by Kurtzberg *et al.*,[149] no patient developed grade IV acute GVHD or experienced hepatic involvement or died of acute GVHD, and only 4 out of 65 patients given an unrelated CBT reported by the *Eurocord Transplant Group* showed grade IV acute GVHD.

From the data collected up to now, therefore, it clearly appears that CBT, from both familial and unrelated donors, is associated with a reduced risk of acute and chronic GVHD.[148-150] In view of this observation, different centers tend to adopt less intensive schemes of GVHD prophylaxis. Typically, children transplanted with a CB unit collected from an HLA-identical sibling receive GVHD prophylaxis consisting of CsA alone, whereas for patients undergoing unrelated CBT the most widely used regimens are those based on a combination of CsA with either low- or high-dose steroids.[149,150] The association of CsA with *short-term* methotrexate as proposed by the Seattle group in BMT recipients[155] is not generally employed due to concerns about the prolongation of time required for engraftment and possible damage to hematopoietic progenitors with a reduction in the potential for marrow repopulation. Procedures involving T-cell depletion of CB cells are also discouraged.

The reported low incidence of GVHD[7,148-150] might, on the other hand, be a major drawback to the use of CB as a source of stem cells for allogeneic transplantation in leukemic patients. In fact, since the role of allogeneic lymphocytes in the control and/or eradication of malignancy is well established, the potential absence of GVL activity could represent a theoretical concern in leukemic subjects given CBT. Currently available data do not conclusively establish whether CBT really predisposes patients to an increased risk of leukemia relapse. However, considering the concern mentioned above, the choice of less intensive GVHD prophylaxis schemes could represent a possible means for partially sparing the immune-mediated GVL effect, which may significantly contribute to preventing regrowth of leukemia cells.

Immunological reconstitution following cord blood transplantation

Although immunological reconstitution after BMT has been extensively studied,[58,59] few data are available on the kinetics of immune recovery in CBT recipients.[144,153,156] After CBT, recovery of T-cell immunity, as well as that of natural killer subpopulations, mimicks what is described in BMT recipients.[153] In particular, in the early post-transplant period recovery of $CD8^+$ lymphocytes seems to be faster than that of $CD4^+$ cells, determining a characteristic inversion of the ratio between the two subpopulations during the first 6 months after CBT, similarly to what is described in BMT recipients. Considering the much lower number of lymphoid cells transferred with CBT as compared to BMT, the recovery of T-lymphocyte number and function towards normal must be considered rapid. The prompt recovery of T-cell immunity following CBT could be positively influenced by the reduced incidence and severity of both acute and chronic GVHD, which *per se* adversely affects the acquisition

of lymphocyte function. However, it must be noted that the prompt recovery of lymphocyte function *in vitro* does not necessarily correlate with effective *in vivo* immunity. In fact, at present there are insufficient data to prove that this rapid T-cell recovery translates into a low incidence of viral and fungal infections after CBT.

In contrast to what is observed in BMT recipients,[58,59] an impressive increase in the percentage and absolute number of B-lymphocytes, apparently not related to viral infections, has been documented in children receiving CBT.[153,157] Possible hypotheses to account for this observation could involve the physiological characteristics of B-cell ontogeny in the first year of life and/or different distribution of mature memory lymphocytes in bone marrow and CB.[158,159]

Ethical problems of cord blood transplantation

Like any other innovative treatment, CBT also poses some ethical questions that have still not been completely resolved. In particular, these ethical considerations can be subdivided into those concerning transplants between HLA-compatible siblings and those regarding CBT from unrelated donors.

The two main ethical problems regarding transplantation from a family donor are those of conceiving a sibling with the hope of producing a compatible donor for a previous child who requires transplantation of hematopoietic stem cells, and of his/her HLA typing *in utero*. Of course, any decision to conceive a child for the sole purpose of making it become a cord blood donor entails belittling the value of the individual to be born. However, it cannot be ignored that it is extremely difficult to separate the reasons that lead to conceiving a child solely for the joy of procreating from those linked to the possibility of saving a living, sick child. On the other hand, even this last reason does not lessen the importance of the future child who will bring happiness to the family in addition to being the person who, in the case of successful transplantation, allowed the family to save the life of a child who would have otherwise been lost.[160]

In the meantime, it is important to stress the inappropriateness of performing HLA typing *in utero*; because of the increased abortion rate due to the procedure (about 1-2%), it entails the risk of causing the death of a healthy human being and would in any case be deeply despicable if it were used to dispose of a conceived child found to be HLA-incompatible with the sick patient. From the point of view of the unborn child, HLA typing *in utero* quite obviously poses critical problems and offers no advantages, but only tangible risks for that unborn child's survival. HLA typing *in utero* should be carried out only when other, far more important reasons (for example, advanced age of the mother with consequent higher risk of chromosome-21 trisomy for the fetus) suggest performing prenatal diagnostic procedures.

Since the donor is a newborn infant, the use of cord blood for an unrelated sick patient has raised many questions of ethical interest. These ethical aspects go beyond the scope of this review, but we would like to comment briefly on some of them. Particular attention has been devoted to the problems raised by tests required to determine whether cord blood is suitable and usable without the risk of transmitting to the recipient any disease carried by the donor cells (namely infectious diseases and genetically transmissible disorders). In fact, for this specific aspect the ethical question is: what kind of behavior should be adopted by the medical operator who works with a woman (or with the parents of a child) if a disease for which there is no therapy is detected in the infant?[161] Such possibly dramatic news must cause as little damage as possible. We must by all means prevent our increasingly profound biological awareness of our selves from leading to a culture of anguish. One might recall in this regard that, for example, it has been stated that minors should not be tested for abnormal genes unless there is an effective curative or preventive treatment that must be instituted early in life.[162]

Another heavily debated problem regarding unrelated transplants is the case of a cord blood unit assigned to allotransplantation, making these cells unavailable in the case the donor needs them for an autologous transplant. However, to ensure that every CB donor has the right to use the donated blood for himself if necessary, there would be no way to provide cord blood units for allotransplants. Therefore the very nature of the technique originally conceived for allotransplants would be profoundly transformed, and this would punish all *donation ethics* at their very core.

Strictly linked to these considerations is the problem of private banking of cord blood cells.[163] In any case, we firmly believe that involving money-making aspects in CB transplantation technology is unacceptable. In particular, as stated by other authors as well,[164] no part of the human body should be commercialized and CB should not be used for the benefit of financial speculators.

Rational use of PBSC in the treatment of leukemic relapse after allogeneic transplant

The cure rate of patients receiving an allogeneic transplant for hematological malignancies is negatively affected by relapse. The incidence and time of disease recurrence depend on several factors such as diagnosis, disease phase at the time of transplant, conditioning regimen, GVHD prophylaxis and T-cell content of the infused graft.[165]

The response rate and clinical outcome of relapsing patients with acute leukemia treated with chemotherapy alone are extremely poor.[166,167] Interferon therapy significantly prolongs the survival of CML patients relapsed after transplant, but its benefit is not durable over long-term follow-up.[168-170] Finally, a second transplant offers some possibilities of cure for relapsed patients but it carries high morbidity and regimen-related mortality.[171-173]

During the past few years the therapeutic approach to post-transplant relapse has been substantially modified. Following its first report by Kolb *et al.*,[174] donor lymphocyte infusion (DLI) is currently being used as a form of adoptive immunotherapy for patients with hematological malignancies who relapse after transplant[175-188] or develop EBV lymphoproliferative disorders.[72,189]

A number of observations in allogeneic transplants support the evidence that a GVL effect, whether associated or not with GvHD and mediated by donor immunocompetent cells, contributes to the eradication of the neoplastic clone.[82,190-195]

The rationale for the use of DLI in post-transplant relapse is based on two main factors: 1) the persistence of an immunotolerant status versus donor cells in the relapsing host; 2) the cytotoxic activity exerted by HLA-unrestricted NK and LAK cells or by HLA-restricted T-cells of donor origin against host malignant cells.[188,196]

However, the effectiveness of DLI therapy is variable since it greatly depends on the type of disease and its stage at the time of relapse. Following DLI, a high proportion of CML patients with molecular, cytogenetic or chronic phase hematologic relapse will likely experience a long-term disease free survival, but the success rate is substantially lower in recurrent AML and virtually absent in patients relapsing with ALL or blast crisis CML.[186] Furthermore, the therapeutic success of DLI is counteracted by related severe complications such as GVHD and myelosuppression, which occur in up to 90% and 50% of cases, respectively. The mortality due to DLI may approach 20%.

Therefore in order to optimize adoptive immunotherapy with DLI and to improve the general management of post-transplant relapse, several biological and clinical conditions should be considered.

One of the most important factors is the time required for GVL to destroy the host neoplastic cells. In early stage CML this time seems to be enough to allow a GVL reaction to build up and eliminate residual CML cells. By contrast, neoplastic growth is so fast in acute leukemia that it may not be challenged by the GVL effect.

A further variable influencing the response to DLI is the potential of the neoplastic clone to mature and differentiate into dendritic cells, which contribute to the GVL reaction by enhancing the antigen presentation capacity of tumor cells. This property is spontaneously attained by CML cells in chronic phase and, to some extent, by AML cells, particularly when cell differentiation follows the tumor reduction induced by chemotherapy.[197] Dendritic cells derived from bone marrow or produced *in vitro* by $CD34^+$ cell cultures in the presence of cytokines[198,199] can exert their action through an HLA restricted mechanism.[200] Therefore, in treating leukemia relapse weakly expressing HLA or tumor-specific antigens, donor hematopoietic progenitor cells may improve the immunologic effect mediated by DLI.

Finally, bone marrow chimerism detected by PCR prior to DLI may predict either response to treatment or the occurrence of myelosuppression. Although long-term persistence of donor T-cells in the peripheral blood during relapse has been reported,[184] this observation does not provide any prognostic information on the post-DLI clinical outcome. Southern blot RFLP analysis, erythrocyte phenotype and cytogenetics have been employed to detect residual donor cells, but no correlation was found among pre-DLI BM chimerism, response to treatment and the risk of myelosuppression. However, pre-DLI BM chimerism assessed by quantitative PCR of VNTR sequences in relapsed CML patients is associated with cytogenetic and molecular remission and strongly predicts the development of aplasia, thereby providing an early indication for the reinfusion of PBSC from the donor.[201,202]

Donor PBSC reinfusion has frequently been adopted as from rescue of DLI-associated myelosuppression. As to the combined use of donor PBSC and DLI, the reported experiences are limited to a small number of patients who relapsed with acute leukemia.[203-205]

In these studies, donors were stimulated with G-CSF at doses ranging from 2.5 mg/kg for 10 days to 16 mg/kg for 5 days. The apheresis products obtained over 1 to 3 consecutive days contained a median of 4×10^8/kg $CD34^+$ cells and a median of 3.5×10^8/kg $CD3^+$ cells. All patients received chemotherapy prior to PBSC infusion and most of them achieved CR with prompt hematopoietic reconstitution which in the cases analyzed originated from donor cells. The majority of patients developed acute or chronic GVHD and related complications. In one of the reported series, the median duration of CR after this combined treatment was longer than the median time from transplant to relapse.[206] These results compare favorably with those recently reported in patients receiving DLI alone for relapse of acute leukemia or myelodysplasia after BMT.[187] Of the eight patients receiving this treatment, only one achieved CR and 7 died of progressive disease.

In conclusion, these preliminary experiences suggest that patients relapsing with acute leukemia or advanced phase CML after BMT should be treated

with intensive chemotherapy regimens, not necessarily including immunosuppressive drugs, followed by donor mobilized PBSC.

This approach might result in certain therapeutic advantages such as: 1) reduction of the tumor burden; 2) slowing down of the neoplastic growth; 3) acceleration of donor hematopoiesis recovery and promotion of dendritic cell differentiation; 4) the possibility for the immunocompetent donor cells to express their GVL activity to a greater extent. Whether the additional administration of cytokines (IFN, IL-2, G-CSF, GM-CSF) would improve the efficacy of chemotherapy and PBSC is unknown at present and awaits further investigation.

Finally, the GVL reaction exerted by donor lymphocytes against CML cells which retain biological features of early stage disease is potent enough that patients might be spared a repetition of previous chemotherapy. However, donor PBSC infusion should be considered for CML patients with either cytogenetic or chronic phase relapse who show minimal (< 10%) or no BM chimerism.[206] In such cases the use of donor PBSC is mainly indicated to counteract the risk of severe BM aplasia following the infusion of DLI alone.

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All authors equally contributed to the conception and writing of this review article.

Disclosures

Conflict of interest: this review article was prepared by a group of experts designated by Haematologica and by representatives of two pharmaceutical companies, Amgen Italia SpA and Dompé Biotec SpA, both from Milan, Italy. This co-operation between a medical journal and pharmaceutical companies is based on the common aim of achieving an optimal use of new therapeutic procedures in medical practice. In agreement with the Journal's Conflict of Interest Policy, the reader is given the following information. The preparation of this manuscript was supported by educational grants from the two companies. Dompé Biotec SpA sells G-CSF and rHuEpo in Italy, and Amgen Italia SpA has a stake in Dompé Biotec SpA.

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