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Placental/umbilical cord blood transplantation

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ABSTRACT

In this article we summarize the clinical outcome of unrelated placental/umbilical cord blood (CB) transplantation, discuss the biological characteristics of CB hematopoietic progenitor/stem cells (HPC) and balance the relative advantages and disadvantages of this therapy as compared with transplantation of other HPC sources. Moreover, we discuss CB banking programs at local, national and international levels.
The data reported by the investigators of the New York Placental/Umbilical Cord Blood Program and of the Eurocord group indicate that the clinical outcome of allogeneic unrelated CB transplantation is significantly related to cell dose, being more effective in children than in adults, and is highly dependent on disease stage at transplantation. Furthermore, both studies show lower graft-versus-host disease (GvHD) frequency and severity and prolonged time intervals for platelet engraftment as compared to those of bone marrow and mobilized peripheral blood recipients. Although the data from the New York Placental/Umbilical Cord Blood Program seem to support a negative effect of HLA differences, the latter were not significantly associated with survival in the Eurocord series. Additional observations are therefore necessary to collect conclusive evidence in this regard.
Currently available data show that CB contains a higher proportion of primitive HPC and that CB-HPC possess higher proliferation and expansion potentials as compared to adult bone marrow. Furthermore, there is some evidence indicating that CB-HPC are more adequate than HPC from other sources for genetic manipulation and gene therapy. Despite the significant advances in the knowledge of the biology and in the clinical use of CB, a number of problems remain unsolved, including the standardization of banking procedures and unit quality and the development of suitable protocols for transplantation of adult patients.

Key words: bone marrow transplantation, umbilical cord blood, placental blood

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Transplantation of hematopoietic progenitor/stem cells (HPC) from bone marrow and mobilized peripheral blood is a standard of care in a number of malignant and non-malignant conditions.[1] Despite continuous improvement, this therapy still suffers from important limitations, including the lack of suitable donors fully matched for the human leukocyte antigen (HLA) system for approximately one third of candidates[2] and high toxicity. The latter relates to (a) the early complications, mainly caused by endothelial damage and represented in its most dramatic expressions by hepatic veno-occlusive disease,[3,4] idiopathic pneumonia syndrome[5] and thrombotic microangiopathy/hemolytic uremic syndrome;[6] (b) the high incidence and severity of acute and chronic graft-versus-host disease (GvHD), which can nonetheless offer protection against malignant relapse;[7-9] (c) the risk of infections in the aplastic period, in the acute GvHD phase and in the late period;[10,11] and (d) the late complications affecting different organs and systems.[12-15]

The discovery that placental blood, also known as umbilical-cord blood (CB), contains high numbers of HPC[16,17] and the success of the first transplant performed in 1988 in a Fanconi's anemia patient[18] prompted the development of large CB banking programs in New York, Düsseldorf and Milan in 1993 and in other locations during 1993-1998. The experience collected in approximately 1,000 CB transplants so far performed has been reported in the literature[19-21] and at international scientific meetings.[22]

In this article we summarize the clinical outcome of unrelated CB transplantation, discuss the biological characteristics of CB-HPC and balance the relative advantages and disadvantages of this therapy as compared with transplantation of other HPC sources. Moreover, we present details of CB banking programs organized in Italy and at the international level.

Clinical results of unrelated cord blood transplantation

The two largest patient series that have been analyzed in detail were collected by the *New York Placental/Umbilical Cord Blood Program* (562 patients)[21] and by *Eurocord* (331 patients).[22] The main characteristics and outcomes of these patient series are reported in Tables 1 and 2 respectively. A comparative compila-

Table 1. Main features of NY-PCBP and Eurocord studies.

Study	*NY-PCBP*	*Eurocord*
Time of transplant	1993-1/1998	10/1988 – 9/1998
No. of cases	562 unrelated	216 unrelated, 115 related
Recipients' age	82% <18 years old	82% <15 years old
Recipients' body weight	All: median 30 kg (<10-116)	Children: median 19 kg (5-50) Adults: median 56 kg (35-90)
No. of banks releasing unrelated units	1 (NY-PCBP)	8 (94% units from NY, Düsseldorf, Milan, Barcelona)
No. of transplant centers	98 in 18 countries	90 in 27 countries
Recipients with leukemia/lymphoma	67%	63%
Leukemics with advanced disease	34%	35% (children)

Table 2. Some outcomes of NY-PCBP and Eurocord unrelated transplants.

Study	*NY-PCBP*	*Eurocord*
Patient group	All unrelated cases (n=562)	102 unrelated children (72 with malignancy)
Neutrophil engraftment	81% by d 42 (median d 28) (500/μL)	74% by d 60 (median d 33) (500/μL)
Platelet engraftment	85% by d 180 (median d 90) (50,000/μL)	42% by d 60 (median d 73) (20,000/μL)
Incidence of aGvHD	23% III-IV	37±6% II-IV
Incidence of cGvHD	25%	7%
Relapse in leukemia	26% by 1 yr	n.a.
Survival	49% event-free survival at 100 days (493 cases quoted in ref. #22)	32±6% (2-yr) in malignancy 70±11% (2-yr) in inborn errors

tion of outcomes of unrelated *Eurocord* CB transplants in children and adults is shown in Table 3.

In both groups, more than 80 percent of transplants were performed in pediatric recipients. Approximately two thirds of the recipients suffered from leukemia or lymphoma and one third of leukemic patients were transplanted in an advanced stage of disease. In both series, CB transplants were performed in a very large number of transplant centers scattered in several countries. This prevented the possibility of evaluating the effect of different preparative and prophylactic regimens on transplant outcome. At variance in the two studies was the number of CB banks which released the units used for transplantation. All units came from one bank in the former study, whereas in the latter 8 banks provided the units. In 94% of the *Eurocord* cases, the units were provided by the CB banks in New York, Düsseldorf, Milan and Barcelona. Moreover, while all the 562 recipients of the *New York Placental/Umbilical Cord Blood Program* study were transplanted with units given by unrelated donors, the 331 patients of the *Eurocord* series included 115 cases who were given units from a related donor. Accordingly, the analysis of the Eurocord study was performed separately in related and unrelated recipients.

Table 3. Some outcomes of unrelated Eurocord transplants in children vs adults.

Study	*Eurocord*	*Eurocord*
Patient group	102 unrelated children (72 with malignancy)	42 unrelated adults with malignancy (67% high-risk)
Neutrophil engraftment	74% by d 60	76% by d 60
Platelet engraftment	42% by d 60	
No. of days to 500 ANC	Median 33 (12-60)	Median 35 (13-57)
No. of days to 20K platelets	Median 73 (9-159)	Median 70 (30-130)
Incidence of aGvHD	37±6% II-IV	43% II-IV
Incidence of cGvHD	7% (3/46 pts at risk)	33% (4/12 pts at risk)
Survival	32±6% (2-yr) in malignancy 70±11% (2-yr) in inborn errors	7±5% (1-yr) in high risk 36±13% (1-yr) in good risk

The clinical data from the *New York Placental/Umbilical Cord Blood Program*[21] show that times to platelet and myeloid engraftments were significantly related to transplant cell dose. In particular, time to platelet engraftment was prolonged as compared to that shown by published series of recipients of HPC from bone marrow or mobilized peripheral blood.[7] The frequency of transplant-related events, which were defined as the occurrence of death, autologous reconstitution or second graft, was significantly related to cell dose and to recipient age and diagnosis, being lowest in recipients aged two years or less and highest in recipients suffering from severe aplastic anemia. The incidences of grade III-IV acute GvHD and of chronic GvHD were 23% and 25%, respectively. Finally, the frequency of relapse, which was 26% by 1 year in acute leukemia recipients, was significantly related to disease stage at transplantation. The Kaplan-Meier estimate of event-free survival at 100 days was 49%.

The 102 unrelated children (72 with malignancy) of the *Eurocord* series[22] showed similarly prolonged times for platelet engraftment. The incidence of grade II-IV acute GvHD was 37±6%. The 2-year survival was 32±6% in patients with malignancy and 70±11% in

those with inborn errors. When patients were stratified by diagnosis, overall 1-year survival was 70%, 35% and 10% in recipients with inborn errors, malignancies and bone marrow failure syndromes respectively. Factors significantly associated with improved survival were CMV-negative status before CB transplantation and ABO match. These data can be compared to those obtained in the 42 *Eurocord* unrelated adult recipients with malignancy, 67% of whom belonged to a high-risk group, as defined as a disease stage at transplantation more advanced than first or second complete remission in acute leukemia or more advanced than first chronic phase in chronic myeloid leukemia (Table 3). These patients showed similar times to and frequencies of neutrophil and platelet engraftments as the pediatric unrelated recipients, but higher GvHD incidences and lower survivals. In particular, survivals at 1 year were 36±13% and 7±5% in the good risk and high risk subgroups of adult recipients respectively. Favorable factors for survival at one year in adults with malignancies of the Eurocord series were number of nucleated cells greater than 10 million per kg of recipient body weight and good risk disease status at transplantation.

The outcome of unrelated CB transplantation in children with acute leukemia was specifically investigated by Locatelli *et al.*,[23] who examined 40 patients with lymphoblastic leukemia and 20 patients with myeloid leukemia reported to the *Eurocord Registry* during April 1990–December 1997. This patient group included 42 and 18 patients transplanted in good-risk and poor-risk conditions, defined as first or second complete remission, and more advanced disease, respectively. Kaplan–Meier estimates of 2-year event-free survivals in the good-risk and poor-risk groups were 40% and 7%, respectively.

In summary, the data reported by the investigators of the *New York Placental/Umbilical Cord Blood Program* and of the *Eurocord* group indicate that the clinical outcome of allogeneic unrelated CB transplantation is significantly related to cell dose, being more effective in children than in adults, and is highly dependent on disease stage at transplantation. Furthermore, both studies showed lower GvHD frequency and severity and prolonged time intervals for platelet engraftment as compared to bone marrow and mobilized peripheral blood recipients. Overall, the relapse rate and the cumulative proportion of recipients showing engraftment were not significantly different from those which would be expected in a comparable group of patients transplanted with bone marrow or mobilized peripheral blood. Although the data from the *New York Placental/Umbilical Cord Blood Program* seem to support a negative effect of HLA differences, the latter were not significantly associated with survival in the Eurocord series. Additional observations are therefore necessary to collect conclusive evidence in this regard.

The information so far collected is not sufficient to identify firm indications for unrelated CB transplantation as an alternative to other stem cell sources. In general, some data that have been published or presented at major hematology meetings indicate that the outcome of CB transplantation is not favorable in patients transplanted in poor-risk conditions, particularly when the dose of nucleated cells/kg of patient's body weight is below 20×10^6. Until conclusive evidence is available, Gluckman *et al.* on behalf of the *Eurocord-Cord Blood Transplant Group*[22] recommended searching bone marrow donor registries and cord blood banks simultaneously and to make the final decision considering the degree of HLA identity, the availability of the donor, the speed of search, the urgency of the transplant, the number of cells present in CB, donor age, sex, number of pregnancies and CMV status.

Biological characteristics of CB

Main features of CB mononuclear cells

The observations that GvHD is less frequent and severe and that HLA match requirements can be less stringent in CB recipients than in recipients of other HPC sources prompted the execution of extensive investigations on the immunologic properties of CB cells. The main findings of these investigations, which have been extensively reviewed,[22,24-27] indicate that CB lymphocytes have a naive phenotype. However, although CB lymphocytes display impaired early NK and T cell cytotoxicity, secondary activation is not dissimilar to that shown by adult blood lymphocytes.[28] In line with the naivety of the CB phenotype, specific studies on B cells from CB showed that these cells present a high level of empty HLA class II molecules, at variance with the high peptide load of adult B cells.[29] This observation opened the interesting speculation that it might be possible to *"artificially charge empty HLA class II molecules on fetal B cells with synthetic peptides for vaccination purposes"*.[29] A possible interpretation of the decreased frequency and severity of GvHD in CB recipients was offered by Min Lee *et al.*,[30] who found that activated CB mononuclear cells showed reduced expression and production of IL-12, a critical cytokine regulating NK and T-cell functions, as compared to adult peripheral blood mononuclear cells. Two important caveats to drawing firm conclusions just from these data were raised by Cohen *et al.*,[27] who remarked that *"the majority of CB transplants performed to date have been in children and recent data have implied that young patients can tolerate more HLA mismatches than adults"*, and that *"the results which show altered lymphocyte function in CB cells compared mononuclear cells from the placental cord with cells derived from the adult periphery. However, it would be more relevant to assess differences between CB and BM [bone marrow], since it is transplants from these stem cell sources which are performed"*. In this regard, interesting data can be found in the article by Gardiner *et al.*,[31] who reported increased ability of CB cells to induce

necrosis-mediated and apoptosis-mediated cytotoxicity as compared to bone marrow cells. These authors suggested that this feature may have a significant impact on the outcome of stem cell transplantation, "*because it suggests that cord blood may have increased potential for a GVL [graft versus leukemia] effect*".[31]

Frequency of HPC in CB versus adult bone marrow

As compared to adult bone marrow, CB contains a higher proportion of immature colony-forming cells (CFC)[32] and an about 8-fold higher frequency of high proliferative potential CFC (HPP-CFC), a population of primitive progenitors capable of giving rise to large colonies.[33] The two HPC sources show similar frequencies of long term culture-initiating cells (LTC-IC),[34] a population of very early progenitors "*unable to form colonies in semisolid cultures but capable of giving rise to CFC after several weeks in Dexter-type long term cultures*".[35,36]

Immunophenotype of HPC from CB

The CD34 antigen is a well established hallmark of HPC,[37] although recent data suggest that a small subset of cells not expressing the CD34 antigen may represent the earliest precursors of the hematopoietic progeny.[38]

The frequencies of $CD34^+$ cells are about 0.2-1% and 1-3% of nucleated cells in CB and in adult bone marrow respectively.[39] Within the $CD34^+$ cell population, those lacking the CD38 antigen ($CD34^+/CD38^-$) represent a subset of the most primitive HPC, which are present in higher frequency in CB as compared to adult bone marrow (approximately 4% in the former versus 1% in the latter).[40]

The most primitive HPC from CB and adult bone marrow show a similar immunophenotype ($CD34^+$, $CD38^-$, $CD45RA^{low}$, $CD71^{low}$). Moreover, most early HPC express Thy-1 (CD90, an antigen inhibiting cell proliferation), c-kit (a ligand of stem cell factor, SCF), and FLT3 (CD135, a receptor of the early-acting cytokine FL). They also show a low signal when incubated with the fluorochrome rhodamine (Rho). At variance in CB and adult bone marrow LTC-IC is the expression of HLA-DR, which is shown by the former but not by the latter.[36]

In summary, the current evidence indicates that the most primitive HPC present in CB and in adult bone marrow share the following phenotype: $CD34^+38^-45RA^{low}71^{low}$Thy-$1^+$c-$kit^{low}Rho^{low}$. Cells with this phenotype are present in CB at a frequency of approximately 1 per 30,000 nucleated cells. Overall, the proportion of immature HPC seems to be higher in CB than in adult bone marrow.[36]

In vitro and in vivo functional differences of HPC from CB versus adult bone marrow

An important difference between CB and adult bone marrow HPC is the higher *in vitro* proliferation/expansion potential shown by the former. This feature, which may be relevant for *ex vivo* expansion programs aimed at increasing the ability of performing CB transplants in patients of large body size, has been associated with a number of reasons, including: more rapid exit from the G_0/G_1 cell cycle phases and autocrine cytokine production;[41] presence of longer telomeres;[42] lower sensitivity to some hematopoietic inhibitors, such as TGF-β, TNF-α, MIP-1α, INF-α.[43]

The *in vivo* functional differences of HPC from CB versus adult bone marrow have been mainly detected in studies performed with animal models, including the NOD/SCID mouse and the fetal sheep, and in gene transfer/transplant procedures.

In comparison to HPC from adult bone marrow, CB-HPC show greater engrafting and repopulating potentials. This is supported by their ability to engraft and repopulate NOD/SCID mice without post-transplant administration of cytokines,[44] which greatly improves the engraftment of HPC of adult bone marrow origin.[45] Comparative studies have also shown that CB contains approximately 1 SCID Repopulating Cell (SRC, the cell capable of engrafting a SCID mouse) per 1 million nucleated cells, as compared to 1 per 3 million and 1 per 6 million in bone marrow and mobilized peripheral blood, respectively.[46] Of high relevance for long-term hematopoietic reconstitution is the observation that cells from bone marrow of primary mice recipients of human CB-HPC are capable of engrafting secondary recipients.[47]

Despite its usefulness, the NOD/SCID mouse animal model suffers from some limitations, which prevent prolonged observation and complete study of hematopoietic and immunologic reconstitutions.[48] Some of the limitations can be overcome with the fetal sheep model developed by Zanjani *et al.*,[49] which was used to demonstrate the long-term engraftment and repopulating capacity of purified $CD34^+$ cells from CB.

As regards genetic manipulation, some data suggest that CB-HPC may be a better target for gene transfer than bone marrow HPC. Studies by Moritz *et al.*[50] showed that the efficiency of transferring the TK-neo and the adenosine deaminase genes into primitive CB-HPC including LTC-IC was approximately double that of into cells from bone marrow. Although the evidence supporting the clinical application of these procedures is still incomplete, genetically modified CB-HPC have been shown to be able to engraft in neonates with adenosine deaminase deficiency.[51]

In summary, currently available data show that CB contains a higher proportion of primitive HPC and that CB-HPC possess higher proliferation and expansion potentials as compared to adult bone marrow. Furthermore, the current evidence indicates that CB-HPC engraft and sustain hematopoiesis *in vivo* and that CB-HPC are more adequate than HPC from other sources for genetic manipulation and gene therapy.

CB expansion

Because of the limitations generated by the relatively small number of HPC present in CB,[52] a number of protocols have been developed for the *ex vivo* expansion of CB-HPC. Some protocols have been designed not only to increase the number of primitive stem cells, but also to promote some degree of commitment of the megakaryocyte lineage, with the expectation that this may contribute to shortening the tempo to platelet engraftment.

Kohler *et al.*[53] investigated optimal conditions for CB *ex vivo* expansion in regard to the influence of HPC enrichment, role of feeder layers, cytokine combinations and mode of culture vessel agitation. These authors reported that optimum results were obtained with a combination of SCF, Flt-3 ligand and IL-3 and that the addition of megakaryocyte-derived growth and development factor improved the expansion results. Furthermore, they found that continuous rotation of culture vessels did not ameliorate the expansion rate. Optimized expansion of CB mononuclear cells grown in spinner flasks in serum-free medium, a condition facilitating the perspective of clinical applications, was reported by Collins *et al*.[54] A large-scale culture system also based on the use of spinner flasks was developed by Kögler *et al.,*[55] who reported an eight-fold *ex vivo* expansion of LTC-IC after 7-14 days of culture, in the presence of Flt-3 ligand, SCF and IL-3. The effects on defined CB cell subpopulations of 7-days exposure to IL-3, IL-6, G-CSF, SCF and Flt-3 ligand, in the presence of IMDM and 10% FCS, was studied by Rice *et al.*[56] Based on the results of this study, these authors concluded that "*$CD34^+38^-$, $CD34^+Thy1^+$ and $CD34^+Rh123^-$ cells have a limited proliferative response to cytokines, the stem cell component of these populations is largely maintained and [cell] expansion is derived from mature cell populations*".

Very prolonged expansion intervals were investigated by Piacibello *et al.*,[57] who were able to obtain extensive amplification and self-renewal of primitive CB stem cells with an *in vitro* system suitable for expanding over 200,000 fold LTC-IC after 20 weeks of liquid culture in the presence of thrombopoietin and Flt-3 ligand. The synergistic effect of IL-6 and IL-11, which contributes to the maturation of the megakaryocyte lineage, was investigated during prolonged culture intervals by Lazzari *et al.,*[58] who identified optimized serum-free and stroma-free conditions for the expansion of $CD34^+$ cells purified from CB, in the presence of thrombopoietin, Flt-3 ligand, IL-6 and IL-11.

The differences observed in these studies indicate that at the present time a universal consensus on the most convenient and appropriate system for the *ex vivo* expansion of CB-HPC is lacking and that research on this topic is still in its infancy. Nonetheless, it is also evident that major strides have been made since the onset of these investigations, as suggested by a number of pre-clinical studies showing the ability of expanded CB cells to engraft immunodeficient animals such as NOD/SCID mice,[57-59] although some concern may be generated by the recent findings of Güenechea *et al.*,[60] who reported an impaired short-term repopulation of NOD/SCID mice transplanted with CB cells *ex vivo* expanded for 6 days in the presence of IL-3, IL-6 and SCF, as compared with the repopulation obtained with fresh CB. It is possible that the diverging conclusions of some studies are mainly related to the different culture systems and cytokine cocktails used for CB expansions.

Some expanded products have already been used for clinical transplantation in humans, although only in investigational protocols.[61-63] In some studies, a device specifically developed for the clinical use of products expanded in a closed, automated system under good manufacturing practice conditions was used.[64] These initial studies were designed with the main aim of testing the safety and tolerability of the administration of expanded cells, which was generally uneventful. Despite the encouraging preliminary results, long-term evaluations of expanded CB recipients will be needed before firm conclusions can be drawn on the clinical impact of this novel form of transplant.

Placental blood banking programs

The CB banking process includes donor selection, CB collection, characterization, cryopreservation, CB unit storage, search and release for transplantation.

Clinicians who search for compatible units for their patients need to be able to access large inventories easily without repeating multiple searches at several hubs and to receive units of pre-defined, high quality levels regardless of the location of the procuring bank.

In turn, the banking and transplantation programs should be operationally connected so as to ensure optimal quality, effectiveness and efficiency. Furthermore, the banking programs should be operated within sound financial plans, as CB procurement and transplantation are associated with very high costs.

In this section we describe the main features of and banking process at the Milan Cord Blood Bank. Furthermore, we present the Italian GRACE and the international NETCORD networks of CB banks, which were developed to satisfy the clinicians' needs discussed above.

Milan Cord Blood Bank

The Milan Cord Blood Bank (MCBB) is located at the Centro Trasfusionale e di Immunologia dei Trapianti of Ospedale Maggiore, Milan, Italy.[65] The current target of the MCBB is to develop an inventory including 5,000 CB units for allogeneic unrelated transplantation and CB donations banked by family-related donors for patients suffering from conditions that may be treated with HPC transplantation. MCBB collections started in 1993 in three delivery

suites. Activities were more recently expanded to include 14 collection sites located in Milan and in other cities at a maximum distance of 80 km.

During 1993-April 1999, a total of 7,309 unrelated and 101 related CB units were collected. Of these, 2,912 unrelated and 101 related units were banked. During the same period, 67 and 9 units were used in Europe, US, Australia and China for unrelated and related transplantations, respectively.

The MCBB operates with a Quality System developed in agreement with the ISO 9002 standard, which was described in detail elsewhere.[66] The Quality System of the MCBB was awarded a certificate of approval on July 18th, 1997, after having been assessed by a third party organization and found in accordance with the requirements of the quality standards EN ISO 9002: 1994. This certificate has been confirmed at regular assessments performed every 6 months.

The CB banking process at the MCBB is performed as described below.

Before proceeding with CB collection, the midwife or the physician or an operator from the MCBB checks for the absence of the following donor exclusion criteria: duration of pregnancy less than 35 weeks; fever in the mother; malformations in the newborn; evidence or suspicion of the presence of hereditary conditions in the newborn; risk behavior or positive serology in parents. Before CB harvest, the operator asks for oral consent from the mother, while informed written consent is collected within 24 hours of delivery. By undersigning the consent, the mother agrees to CB harvest, banking and allogeneic transplantation for unrelated recipients, to serologic testing at delivery and at a later check performed six months after delivery, to biologic material storage, retrieval and testing if necessary, to personal data storage on paper and in electronic format and circulation in anonymous format.

CB is collected by midwives in a plastic bag containing CPD as an anticoagulant, by means of puncture of the umbilical vein after accurate disinfection of the cord. Midwives are trained to perform collections and regularly audited for this activity by MCBB staff. Collections are performed with the placenta *in situ* in vaginal deliveries and after placental delivery in Cesarean sections. Time of umbilical cord clamping varies in the delivery suites, due to the lack of a prevailing opinion in regard to the correct time of clamping (early versus late).

CB units are stored at +4°C during transportation to the MCBB. Generally, a member of the MCBB staff transports the units from the delivery suite to the bank. Units collected in delivery suites outside Milan are transported by car or by train. Transportation is performed in insulated containers similar to those used for transportation of red cell concentrates. Units are accepted at the bank if there is perfect match of data on sample tubes, bag and paper forms.

Units with a volume below 60 mL and total white cell counts below 800×10^6 are not processed for banking. Processing consists of volume reduction with a bottom-and-top procedure[67] and cryopreservation with DMSO at 10% final concentration.[68] The latter is performed only with volume-reduced units showing white cell counts greater than 600×10^6 prior to cryopreservation. Units are stored in the liquid phase of liquid nitrogen tanks.

At time of banking, a sample of CB is used to perform a complete blood count, sterility tests for aerobic and anaerobic bacteria, CFU-GM and $CD34^+$ cell counts, ABO/Rh, HLA-A,B serologic typing, HLA-A,B DRB1 molecular typing at low resolution. A maternal blood sample is used to perform the serologic screening, which includes detection of HBsAg, anti-HIV 1-2, anti-HCV, anti-HTLV I-II, anti-CMV (IgG and IgM), anti-toxoplasma (IgG and IgM) antibodies and TPHA. Serology is performed on the basal serum collected at delivery and on a new maternal sample collected 6 months after delivery. At this time, a physician from the MCBB interviews the mother to collect the baby's medical history. Before unit release, cell viability, clonogenic potential and sterility are determined in a unit specimen obtained from a segment stored under the same conditions of the bag. Moreover, at unit release maternal HLA-A, B, DRB1 typing at low resolution is performed on a maternal sample stored in the frozen repository. Units are transported to the transplant center in an approved dry shipper. Patient's follow-up is performed at regular intervals using the MED-A and MED-B forms developed by the *European Group for Blood and Marrow Transplantation* (EBMT).

An analysis of economic issues performed at the MCBB in 1996 showed that no less than 3% of the bank's inventory should be released per year at an individual fee of US$ 15,000 per unit released to recover fully the costs of bank implementation, unit processing and storage.[69]

GRACE

In 1995 the MCBB proposed the formation of GRACE, the acronym of the *Gruppo per la Raccolta e Amplificazione delle Cellule Ematopoietiche (Group for the Collection and Expansion of Hematopoietic Cells)*, as a common forum for clinicians and investigators interested in CB banking and transplantation.[65] GRACE, which aims at developing a global inventory of 15,000 CB units with similar, high quality standards before year 2002, operates within the *Gruppo Italiano per il Trapianto di Midollo Osseo* (GITMO) and the *Eurocord* program of the EBMT. Besides the cultural implications, GRACE was developed to harmonize banking procedures at high quality standards and to facilitate CB unit searches performed by clinicians through the development of a unique hub able to search the whole inventory of the member banks. Current GRACE individual membership includes 166 physicians (blood

bankers, hematologists, obstetricians, neonatologists, immunologists), biologists, midwives and information technology experts. Institutional GRACE members are the CB banks located in Bologna, Florence, Milan, Pavia, Padua, Rome, Turin. Other banks may apply for membership if compliant with the following rules: a) availability of a local inventory of at least 500 cryopreserved CB units (1,000 for applications submitted after 1999); b) existence of a local quality system developed with ISO 9002 as a model of reference; c) use of procedures approved by the GRACE Board of Directors; d) formal recognition by the Health Authority; e) payment of annual membership fees.

The Quality System managers from the GRACE institutional banks participated in 1996-1997 in a training program aimed at the implementation of local Quality Systems similar to that implemented in Milan,[66] which were developed in 1997-1998. On December 3rd, 1998, the GRACE network including the banks in Milan, Turin, Florence and Rome was awarded a certificate of approval of the Quality System in accordance with the standard EN ISO 9002: 1994. Since that date, the hub at the Milan Cord Blood Bank has been searching units within a unified inventory of approximately 3,500 units stored in the four banks participating in the program. The organizational flow-chart of the GRACE network is shown in Figure 1.

NETCORD

Following the positive experience collected with GRACE at the national level, in 1996 the MCBB proposed a similar program at the international level, which involved, in addition to Milan, the CB banks in Düsseldorf and Barcelona. The program, which was named NETCORD, was formally constituted in 1998, at the end of a pilot phase spent to determine its feasibility.[70] At present, NETCORD founders are refining the minimum product specifications and operational standards. This harmonization is particularly important also in view of the existing documents addressing these topics.[71,72]

The group works with ISO 9000 as a model of reference. NETCORD features are as follows: a) each bank makes its own inventory accessible on line to the other networked banks; b) two banks (currently Milan and Düsseldorf) act as NETCORD hubs. They are able to receive CB unit search requests in electronic and paper format, to explore the global inventory and to provide a search report to the transplant center. The report lists the units ranked by HLA

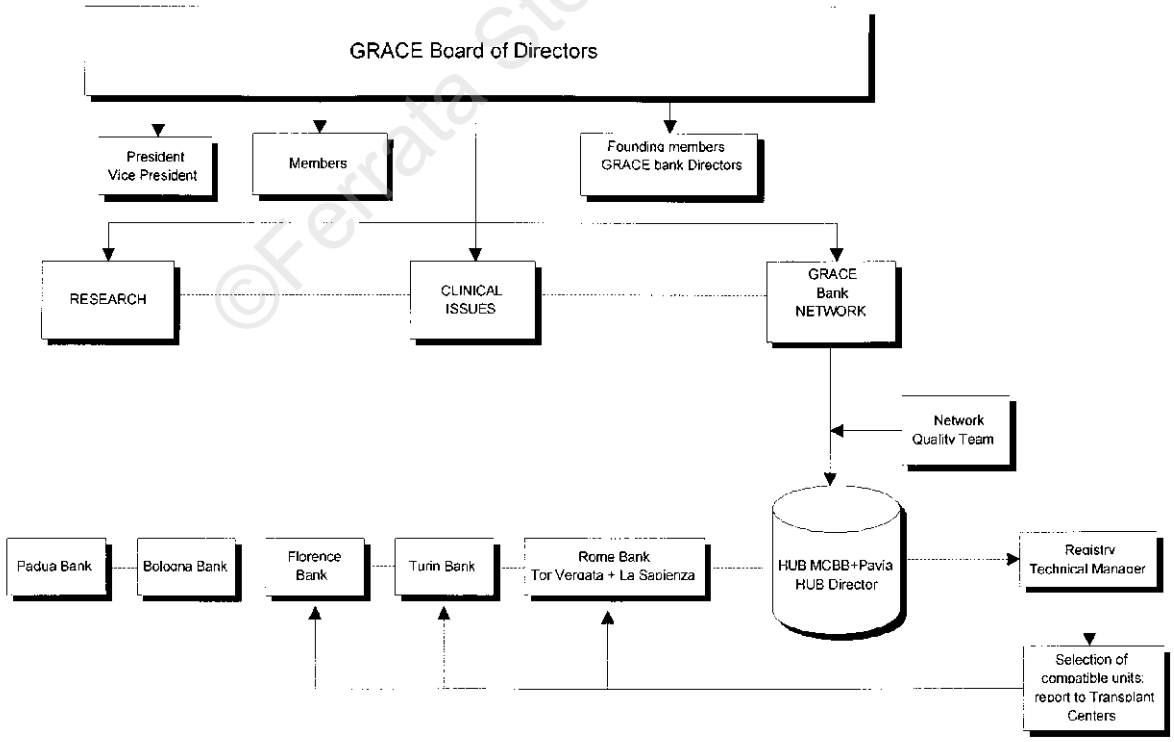

Figure 1. Organizational flow chart of the GRACE network.

match and, within the same match level, by total number of nucleated cells per unit. Further contacts are developed between the transplant center and the bank in which the unit of interest is stored. NETCORD, which can be accessed not only by transplant centers, but also by other networks and organizations, is currently being expanded to other CB banks.

Conclusions

Transplantation of hematopoietic progenitor and stem cells from CB is a novel form of therapy in rapid expansion which, although presently applied only in investigational protocols and mainly in the pediatric population, may represent an alternative source to bone marrow and mobilized peripheral blood in some patient categories.[19-23]

The need to clarify numerous clinical and biological issues before this form of transplantation can represent a standard of care requires the implementation of efficient programs at national and international levels to perform an adequate number of transplants in a short time interval with CB units of known, high quality standards.

A critical issue in this regard is the definition of the number and size of the banking programs that are necessary to match clinical needs of allogeneic unrelated transplantation. In view of the significant and risky investments required for the development of high-quality banking programs, it may be prudent to consider that, of the approximately 15,000 transplants performed in Europe in 1996, about 10,000 and 4,000 used autologous HPC and allogeneic HPC from family-related donors, respectively.[73] The remaining figure of about 1,000 cases annually in need of unrelated donors in Europe may be used to try to define the potential number of annual *customers* of CB banks worldwide in the next few years. One might use the (perhaps optimistic) hypothesis that 30% of allogeneic, unrelated HPC sources may come from CB, i.e. 300 units per year in Europe, with a population of approximately 325 million inhabitants. This would translate into 1 CB unit per million inhabitants per year. In addition, although regrettable, it may be prudent to assume that this expensive form of therapy may not be regularly accessible in the near future to many patients treated outside Europe, North America, Australia and Japan. It thus seems that the large number of CB banks currently present 'on the market' will compete for approximately 1,000 CB 'customers' per year, i.e. 1/1,000 of one billion people. How many banking programs can be supported with the reimbursement fees (currently US$ 15,300 per unit released) generated by 1,000 CB units per year?

Based on the above hypothesis and on a reported estimation by the NMDP of a global requirement of 50,000–100,000 CB units,[74] we believe that the expansion of CB banking outside well co-ordinated national and international programs with very high quality standards should be discouraged. Furthermore, the national communities should be aware that the number of banks should not exceed well defined limits, which have been already reached or surpassed in some areas. In the next few years, it will be the task of the existing programs to contribute to answering the pending questions on the biological characteristics of CB and the clinical indications of CB transplantation.[75]

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