The Era of Umbilical Cord Blood Transplantation and banking

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In allogeneic hematopoietic stem cell transplantation, the stem cells are obtained from a sibling or a volunteer unrelated donor. The donor and the recipient must be fully or closely HLA-matched to reduce the risk of life threatening complications e.g. graft versus host disease or graft rejection. A sibling donor has the best chance to match. The gene for HLA are located on chromosome 6 and, as the inheritance follows the simple Mendelian rule, a recipient has a 1:4 chance of finding complete HLA matched sibling donor. Several sources of stem cells have been explored for potential use in regenerative medicine and hematological malignancies. These are bone marrow, blood stream or umbilical cord blood of newborn babies. More recently, induced pluripotent stem cells (iPS) have been generated from skin fibroblasts, keratinocytes and blood progenitor cells. They seem to reprogram embryonic properties, although still not in use. Over the last 20 years, extensive research has established the safety and efficacy of umbilical cord blood transplantation (UCB) in both children and adults; and, thereby, it has rapidly become a valuable alternative stem cell source for allogeneic hematopoietic stem cell transplantation. It would be extremely useful if iPS cells could be generated from cord blood cells.[1,2,3,4]

Umbilical cord blood as a source of hematopoietic stem cells

The degree and severity of complications and side-effects depends on several factors and many issues must be considered. Ideally an individualized decision is made for each patient.

The availability of HLA matched donors: Hematological malignancy is the most common indication for allogeneic HSCT in both children and adults. In this circumstance, the speed of availability is often critically important. Because of the rapid availability of units, UCB may be a particularly attractive option. In general, 8/8 HLA-matched BM remains the 'gold standard' for alternative donor HSCT. But UCB requires less stringent HLA matching and despite considerable HLA disparity, it is associated with relatively less acute and chronic graft-versus-host disease (GVHD). Therefore, UCB should be considered a reasonable option in those that do not have a matched related or unrelated donor and for those in whom the time to transplant is critical, such that waiting for an matched unrelated donor (MURD) bone marrow would not be in the best interest of the patient. Although a recent analysis shows that treatment related mortality (TRM) is lower and LFS higher when MURD bone marrow and matched peripheral blood stem cell (MPBSC) are used as compared to the other sources, suggesting that these graft sources are preferred when available and time permits.[2]

The size of the patient: In contrast to children, use of UCB in adults has been more restricted due to limitations of cell dose i.e. quantity of the stem cells to be transplanted. An adequate single unit has been defined at the University of Minnesota as: $>3.0X10^7$ nucleated cells/kg for 6/6 HLA-matched units, $>4.0X10^7$ nucleated cells/kg for 5/6 HLA-matched units and $>5.0X10^7$ nucleated cells/kg for 4/6 HLA-matched units. However, partially HLA-matched UCB with an adequate cell dose ($>2.5X10^7$ nucleated cells/kg) is a suitable alternative when an HLA-MURD is not available or when the transplant is urgent. This cell dose is often achievable with a single UCB unit for young children, but not possible for adult recipients. This problem can be solved by co-infusion of two partially HLA-matched UCB units which is safe and efficacious, regardless of the intensity of the conditioning.[2]

The nature of the disease: Hematopoietic stem cell transplantation has curative potential for hemoglobinopathies including sickle cell disease (SCD) and thalassemia and storage diseases. In cases of inherited disorders, HLA-matched sibling donors (MSD) transplantation results in a high survival rate with few transplant-related complications and is an accepted treatment for high-risk disease. HLA MSD must also be free of the underlying genetic disease, making the chance of finding a HLA-matched and healthy related donor even less likely; thereby, appropriately matched alternative

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donors are often used. Because the risks of TRM and GVHD with BM grafts are not insignificant, UCB is an attractive option. Although the use of related UCB is clearly superior over related donor BM due to the decreased risk of GVHD, MURD BM still remains the gold standard with UCB reserved for those without a MURD BM donor. Because the time from diagnosis to definitive treatment is crucial to prevent neurological disease progression and UCB units can be obtained quickly, its use is especially desirable in these patients.[2]

Sex matching: The protein H-Y is coded for by a gene on the Y chromosome and is therefore only expressed on male cells. Female donors, specifically multiparous individuals, may have been primed against such antigens and, as a consequence, have circulating T-cells that can recognize host cells expressing these proteins. This is likely to underlie the increased risk of GvHD in male recipients of female grafts. The pre-harvested umbilical cord blood may solve this type of sex mismatch.[5]

Post transplant infections: Serious infection after MURD transplant continues to be a major problem regardless of donor source. UCBT is associated with longer post transplant median duration of neutropenia and increased risk of bacterial infections in adults early after UCBT, but not in the pediatric population. The risk of dying from infection is the same as the risk after other types of transplantation and there is same incidence of CMV, fungal or other viral infections.[2]

Donor-derived leukemia after alternative donor HCT: Donor cell leukemia (DCL) is a rare complication of allogeneic HCT with an unclear etiology. It has been hypothesized that the incidence may be higher after UCBT when compared to other stem cell sources and the estimated risk is still <1% for UCBT.[2]

New methods for improving results of SCT

Low cell content in the transplanted product is the main factor for graft failure and late engraftment in UCBT. These result in infections during lengthy post-transplant neutropenia and, along with toxicity of preparative regimens, increased early transplant-related mortality. All of these have limited the wider use of UCBT, especially in adults. Another concern with UCBT relates to the possibility of poor development of anti-tumor and adaptive immunity against pathogens. Strategies that have been pursued to obtain high rates of engraftment include, among others, ex-vivo expansion of HSC, intra-osseous infusion, infusion of two UCB units, and the co-infusion of third party donor (TPD) mobilized hematopoietic stem cells (MHSC). All these may be combined with reduced intensity conditioning.[6]

Double cord blood transplant:

Both infused cell dose and degree of HLA matching of cord blood units correlate with engraftment and outcomes. Determining appropriate minimum thresholds is complicated by the interaction between cell dose and HLA typing. The minimum number of nucleated cells should be $3X10^7$/kg or CD34+ cells $2X10^5$/kg.[4] For many adults and large children, no single units are available that meet these cell dose requirements. To overcome this obstacle, the double cord transplant model may be introduced in which two cord units are infused simultaneously to increase cell doses. This technique significantly increases engraftment rates and contributed to decreased TRM. In spite of apparent increase in engraftment rate following double unit CBT, time to engraftment remains similar to modestly reduced compared to the time of engraftment following single unit CBT. Double unit CBT is associated with increased rate of moderate acute GVHD as compared to single unit CBT, although rate of severe acute or chronic GVHD is not increased. It is associated with a decreased risk of relapse, at least for patients with good disease control at the time of transplant.[7]

The biology behind double unit CBT is not well understood. In the vast majority of cases, a single unit emerges as the sole source of long term hematopoiesis. By day 21 post-transplant, single unit dominates in over 80% of patients, and by 1-year post-transplant, in nearly all patients. Some degree of mixed chimerism may be present in a larger portion of patients undergoing RIC conditioning than myleoablative conditioning. No factors, including viability, infused TNC, CD34+, CD3+, sex mismatch, ABO blood group, HLA mismatch, and order of infusion, have been identified to date that reliably predict which unit will emerge as the winner. It is postulated that the 'losing' unit may facilitate engraftment, perhaps by increasing numbers of infused ancillary cells, such as mesenchymal stem cells; or, alternatively, the use of two units may simply increase the chance of infusing a unit with engrafting potential. The immunological interactions may underlie the emergence of a winning unit and the CD8+ T cells may mediate rejection of the losing unit. Each unit is required to have a TNC e" $1.5X10^7$/kg and must be at least 4/6 matched to the patient. Preference is given to better-matched acceptably sized smaller units, and units must be at least 3/6 matched to each other. Because of high variability in assays assessing CD34+ cells, CD34+ counts should not be considered unless

comparing units compared by TNC, then selected with the higher CD34+ count. For patients who have an adequately sized single unit, it is uncertain whether addition of a second unit might improve outcomes.[7]

Reduced intensity conditioning:

Risk of TRM has limited the use of alternative donor HSCT in elderly patients, in those who have been heavily pretreated and/or come to transplant with co-morbidities. Various non-myeloablative or reduced intensity conditioning (RIC) regimens have been used as an alternative to myeloablative conditioning (MAC). The purpose of RIC is to take advantage of the graft-versus-leukemia effect of the immunocompetent cells in the graft, rather than the antitumor effect of high-dose chemo-radiotherapy. The goal is to provide sufficient immunosuppression to prevent graft rejection, to achieve and maintain complete chimerism and to promote a graft-versus-malignancy effect. Although there was higher graft failure and delayed neutrophil and immune recovery after UCBT in the myeloablative setting in previous study, recent study encourages for UCBT in the RIC setting. The proposed conditioning regimen by Minnesota group consists of Fludarabine (200 mg/m^2), cyclophosphamide (50 mg/kg) and a single fraction of 2 Gy total body irradiation with ciclosporin and mycophenolate mofetil for post-transplantation immunosuppression. Favorable risk factors for survival were absence of high-risk clinical features (Karnofsky 50–60, serious organ dysfunction, recent fungal infection) and absence of severe GVHD, and favorable risk factors for EFS were absence of high-risk clinical features and use of 2 UCB units.[2,4]

Intra bone infusion:

Intravenously injected stem cells recirculate and only a small fraction of stem-cells migrate and home to hematopoietic sites; but direct intra bone injection of hematopoietic stem cells resulted in 10-times more efficient repopulation of the marrow of lethally irradiated mice and also proved in limited human study. A single cord blood transplant injection directly in the iliac crest of leukemic patients after standard myeloablative conditioning shows no complication during the infusion, faster neutrophil and platelet engraftment and very low incidence of severe GVH effect.[4]

Third party donor (TPD) transplants/dual transplant:

Recipients of UCBT have long-lasting post-transplant periods of deficient adaptive immunity. Post-transplant recovery of natural killer cells occurrs soon after the transplant and B cells recover around 6 months, but T-cell recovery took more than 1 year. T cell recovery derives from UCB–HSC through thymic differentiation and that cytomegalovirus (CMV)-specific lymphocytes develop following CMV reactivations. Because the NK cells are important effectors of GVT effect, this pattern of cell recovery is responsible for high GVT effect and low incidence of serious GVHD. This could have interesting implications regarding the selection of the TPD. In this strategy, UCBT are used with co-infusion of a limited number of highly purified mobilized HSC from a HLA unrestricted TPD. Shorter post-transplant periods of neutropenia are usually observed in UCBT with relatively low cell content and 0–3 HLA mismatches after myeloablative conditioning. This results from an early and initially predominant engraftment of the TPD mobilized HSC. After a variable period of double complete TPD+UCB chimerism, final full UCB chimerism is achieved within 100 d(cumulative incidence >90%). Early recovery of the circulating neutrophils reduces the incidence of serious neutropenia-related infections. It also facilitates the use of drugs with myelosuppressive side effects to combat other complications. The incidence of GVHD and relapses is also low, with overall and disease-free survival curves comparable to those of HLA identical sibling transplants. The donor NK cells should be killer cell immunoglobulin-like receptor (KIR) incompatible with the recipient but compatible with the transplanted CB unit to improve the anti-leukemic and anti-GVHD effects.[6]

Use of other TPD cells in patients recipients of 'dual transplants':

The unavailability of the donor for additional donations is a disadvantage of UCBT which could be overcome by using different cell types from TPDs for further cell therapy actions. Donor cells that may have the capacity of producing preventive or therapeutic effects include NK cells, mesenchymal stem cells (MSC), pathogen or tumor-specific cytotoxic T-lymphocytes (CTL) and T-reg lymphocytes.[6]

NK cells: NK cells play a significant role in reducing GVHD and eradicating residual disease after HSCT. These actions are the result of cytotoxic effects of donor NK cells on recipient antigen presenting cells and leukemic cells when the NK cells are not inhibited by ligands to the KIR (killer cell immunoglobulin-like receptor) and other receptors that recognize class I HLA molecules of the recipient. Peripheral blood NK cells (CD3-depleted lymphopheresis products) have also been used for adoptive immunotherapy in chemotherapy refractory patients.[6] Regarding their capacity to kill malignant cells, UCB mononuclear cells

(MNCs) have less cytotoxicity compared to PB MNCs, but they rapidly respond to cytokine stimulation, resulting in killing that is not different from similarly treated NK cells from adult PB.[8]

MSC: Mesenchymal stem cells has immunosuppressive properties regarding prevention and treatment of GVHD in case of skin engraftment, but these effects are not established in HSC transplant and are now under evaluation to improve engraftment and GVHD.[6]

Viral specific CTL: In vitro expanded virus-specific cytotoxic T-lymphocytes are attractive option for prophylaxis and/or treatment of post-HSCT viral infections. Researches are ongoing to simplify the process of obtaining products suitable for treatment of CMV reactivations and infections.[6]

Tumor specific T-cells: The lymphocytes play a major role in the rejection of leukemic cells. Tumor antigen-targeted immunotherapy may be an efficient procedure to eliminate residual tumor stem cells that may persist as a reservoir of primitive and chemo-resistant tumor cells. T-cell therapy requires distinguishing GVT and GVHD effects to allow the reconstitution of adoptive immunity against infectious agents and also antigenic structures expressed by tumor stem cells. EBV-associated post-transplant LPD is a highly immunogenic tumor that may prove amenable to control by TPD virus-specific CTL. Cell products with a sufficient number of highly enriched tumor-specific T cells are likely to be devoid of significant numbers of allo-reactive T cells carrying the risk of GVHD, but products of this quality are difficult to produce. [6]

Gene therapy: The incorporation of suicide genes that allow the selective destruction of allo-depleted or antigen-selected cells after infusion is another approach to increase the safety and potential applicability of these therapies to patients. It is conceivable that these kinds of T-cell immunotherapy products derived from voluntary donors could prove useful for prophylactic or therapeutic use in the setting of UCBT.[6]

T-regs (CD4+25+ T regulatory cells): The possibility of using donor T-regs as a tool to modulate immune reconstitution to a favorable balance between development of adaptive immunity, the preservation or enhancement of GVT effect and minimization of GVHD risk, is presently under investigation.[6]

Embryonic/induced pluripotent stem cells and non-hematological uses of UCB

For 15 years cord blood has been used interchangeably for bone marrow in the stem cell transplantation of various malignant and genetic blood diseases. Regenerative medicine applications are different from typical stem cell transplants as they do not require the severe pre-conditioning regimes and generally require the use of autologous stem cells for the therapy to be successful; otherwise, immune rejection will usually occur. Embryonic stem (ES) cells have the ability to develop into any tissue in the body and their uses are often promoted as the optimal stem cell source for regenerative medicine. There comes a need to create patient-specific (i.e. 'tissue-matched') ES cells either by therapeutic cloning or by use of induced pluripotentcy methods (i.e. induced pluripotentcy stem (iPS) cells). Although the threat of immune rejection can be overcome in this fashion, the threat of teratoma formation is omnipresent in any type of ES cells when these cells are directly used in patients without first differentiating the ES cells into the desired tissue which involves large amounts of time and monies. The creation of patient specific ES cell lines require months and even the newly created patient-specific ES cells may be unable to differentiate into required cell lines or tissues. Thus, ES-based therapy has significant limitations, because most patients present with a limited time to treat, as in heart attack or a stroke. Moreover, it is unfortunate of limited applications of ES and iPS due to ethical, political, biological and regulatory hurdles. The overall costs may be the major limiting factor for the progress of ES cell therapies.[1]

Generally, tissue-derived stem cells have limited self-renewing capabilities and are unable to reconstitute a whole organ system. However, CB stem cells have an advantage over other sources of stem cells, because they are unique in their ability to undergo pluripotential differentiation into cells of endodermal, mesodermal and ectodermal lineages; the supply is unlimited; they can be used in autologous or allogeneic situations; they need minimal manipulation; and they raise no ethical concerns. Thus, UCB appears to be a practical and readily available substitute for ES cells in many of the same applications, and their use is rapidly expanding in regenerative medicine that surpasses the standard use in stem cell transplant. Currently, UCB stem cells are being used to treat stroke, spinal cord injury, cerebral palsy, traumatic brain injury, juvenile diabetes, hearing loss and corneal and skin injury.[1]

Cord blood banking

In the absence of a suitable sibling donor, the treatment of choice is a HLA-matched volunteer unrelated donor, optimally having at least nine out of 10 high resolution (HLA-A, -B, -C, -DRB1 and -DQB1 matches. At present, approximately only 30% of patients can find an HLA

identical donor from within the family.[9] However, the success rate of donor searches is, at best, 80% after 6 months with a median time duration of 2 months in extremely large and efficient bone marrow registers and is far from being achieved in countries where the ethnic background is more diverse. With the reduction in family size and more and more patients need, the search for a well-matched HLA type unrelated donor even in the established donor registries became more difficult. The umbilical cord blood contained cells are capable of reproducing hematopoiesis in vitro and that these cells could be cryopreserved. The immunological naïveté of the newborn cells and the high expansion potential of its circulating stem cells; the faster availability of banked cryopreserved CB units; decrease in the donor size inventory due to tolerance of 1–2 HLA mismatches out of six and higher frequency of rare haplotypes by targeting ethnic minorities; lower incidence and severity of graft-versus-host disease (GVHD); lower risk of transmitting infections by latent viruses; lack of donor attrition; and lack of risk to the donor – all give advantages to CB.[10] These observation and successful UCB transplant led to the establishment of the first unrelated cord blood bank (CBB) from voluntary donors in New York in 1991. The field of cord blood banking is now well established and has evolved substantially since it was first described. Its evolution has been greatly influenced by the clinical results and by a number of regulatory issues which are now in place. At present there are 54 public unrelated CBBs in different parts of the world with over 300 000 units frozen, hence immediately available for transplantation.[9]

Steps of Cord blood banking

Recruitment and informed consent: Cord blood banking is a very complex and expensive procedure and it is therefore crucial to try to maximize the resources and efficiency of the program. All cord blood units (CBU) collected need to have a signed consent obtained prior to labor, more preferably, at around 30 weeks of pregnancy to increase the efficiency of the collection by decreasing the wastage of CBUs discarded due to the lack of consent. Detailed and clear information should be provided about the tests required, the intended use of the unit particularly in relation to the altruistic nature of the donation, and about the potential use of the clinically unsuitable units for research and development. It is important to select maternity units not only with high numbers of deliveries but also with an ethnically mixed population of potential donor mothers, in order to expand the HLA profile of the banked units.[9]

Cord blood collection: The collections of UCB from full term deliveries can be performed in utero or ex utero. In utero collections are performed by a trained member of the delivery team during the third stage of labor before the placenta is delivered. Alternatively, the UCB can be collected by trained staff ex utero from the freshly delivered placenta, following full term normal delivery or caesarean section. This is carried out by suspending the placenta, cannulating the vein and allowing the blood to drain by gravity into a specially designed UCB collection bag. In ex utero collections, the risk to the mother or infant is minimal, but the risk of microbial contamination could be higher. In utero collections may be associated with larger volumes and total nucleated cell (TNC) doses than ex utero collections, but if appropriately trained staffs are involved, there is no significant difference in the volume, or indeed in the contamination rate, with either of these two methods. The UK Royal College of Obstetricians and Gynecologists and the Royal College of Midwives have recommended that all UCB collections should be made ex utero to avoid the possibility of early clamping and the diversion of the

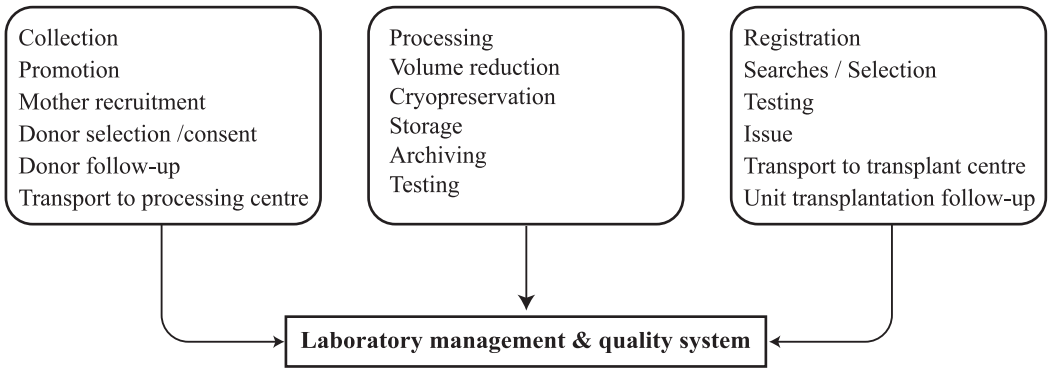

Fig.-1: *Principal elements of cord blood banking*[9]

attention from the mother and newborn to the UCB collection.[9]

Processing: All CBUs should be processed within 24 h of collection in either a closed system or in an environmentally controlled clean room. Long-term storage of large numbers of frozen units may create a space issue which may be solved by reducing the volume of the CBU prior to storage. A number of volume reduction methods have been introduced, the majority of which deplete the unit of red cells and plasma, leaving the buffy coats in a standard volume, whilst maintaining the quality and quantity of the stem cells collected. At present most CBUs are reduced to a standard volume of 21 ml prior to freezing, using automated systems. Long term viability of the frozen cells was also of concern but it is now known that the standard cryopreservation protocols of freezing give an average of 80% recovery of nucleated cells and >90% recovery of progenitor cells. Both the automated and the manual systems of freezing are perfectly adequate, provided the temperatures are regularly monitored and the process is fully evaluated and quality controlled. In order to maximize the volume of cells stored, all the 'waste' components produced during the processing of the units are utilized for testing and archiving. A blood film is prepared from the fresh cord blood to perform an initial hematological screening of the unit and a small piece of cord tissue is collected and frozen as a source of DNA for future testing, if required.[9]

Testing: Some tests need to be performed upfront for banking and for registration purposes, either in the mothers or the CBUs; others can be performed once the unit has either been reserved or selected for transplantation. Amongst the tests required at banking are those performed on the mother's blood, which, in the UK, are the same as those required for blood donors. With the introduction of volume reduction, it is now necessary to perform a full blood count, TNC, nucleated red cell and CD34 counts before and after processing, in order to assess the effect of the manipulation on the viability and quality of the unit prior to its long term storage. The finally processed unit is also tested for both aerobic and anaerobic cultures to assess the presence of bacterial and/or fungal cross-contamination from the birth canal or systemic sepsis in the donor-mother or infant. Bacterially contaminated unrelated units should be discarded.[9]

Registration of CBU: On completion of processing and testing, all the information regarding the mother and the CBU must be reviewed to assess the suitability of the unit for inclusion into the bank. All CBUs are registered under a unique identifier with the following information: HLA type, volume of collected cord blood and TNCC of the final product.[9]

Testing at reservation, selection and issue: When a CBU is reserved or selected for transplantation, a number of additional tests are performed at the request of the transplant centres. The range of required tests for infectious disease markers is expanding and now includes Epstein Barr virus (EBV), human herpes virus (HHV)-6, -7 and -8 and toxoplasmosis. High resolution HLA typing and screening for abnormal hemoglobins in selected cases are also performed prior to the issue of the unit. The request for CFU assays to assess the functionality of the CB cells is still controversial; due to the high cost and lengthy procedure taking up to 14 days, most CBBs perform CFU assays at the stage of reservation of the unit.[9]

Regulations of Cord blood banking

CBB activity involves the import and export of a cellular product across different countries and needs to operate within a highly regulated environment in order to ensure that the donations are safe and meet the highest quality of standards. The regulatory aspects covering the activity of CBB have increased significantly in recent years.[9]

Related and autologous CBB

The banking of directed CBUs, either related or autologous, requires different considerations where the cord blood is collected from the sibling of a patient with a disease that can potentially be treated with a cord blood transplant. In these cases, considerations such as minimum volume collected, volume reduction or exclusion due to microbial contamination during storage do not apply. Most of the banked related units used have been fully HLA-matched for patients with hemoglobinopathies and, in some places, related cord blood transplantation is the first line of treatment for patients with thalassemia major. But up to 70% cases, the collected CBUs are not fully HLA-matched with the patient and those units may not be used for the intended patient; in these cases, the CBUs will have to be kept frozen indefinitely unless clear policies regarding their disposal are put in place. In future, with the possibility of prenatal genetic diagnosis (PGD), it will be possible to collect units selected only from HLA-identical siblings, as has already proved possible.[9]

Establishing a national CB program

A national CB program designed to supply thousands of donations will require the participation of many parents-to-be, the involvement of various hospitals and perinatal care providers and public health campaigns. The practical

implications and limitations should be considered in order to optimize the CB bank to be useful. The factors to be considered to design a national CB bank are- the number of patients that would benefit, the optimal size of a CB bank for the country according to pre-defined and quantifiable targets, the quality and the costs associated with the project. Using available data from developed countries, it seems that using 50 000 high quality units (i.e. acceptance thresholds of $9X10^8$ and, may be, at least $3X10^6$ viable CD34+ cells), with a high ethnic diversity, the National CB program can be reasonably cost-effective. If combined 2 units CBT can be used successfully to treat adults with a high body weight, this national inventory could guarantee an optimal CBT option for three quarters of the patients in need.[10]

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