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General Discussion
25 Years of cord blood transplantation

Since the first cord blood transplantation (CBT) in 1989, over 30,000 CBT have been performed worldwide. Especially in the last 15 years there has been a considerable increase in cord blood transplantation (CBT). Given the minimum dose of cells per kg of recipient bodyweight that is needed for a successful outcome, CBT of a single cord blood unit was initially limited to children. The number of CBT performed in patients older than 16 years has steadily increased and in 2011 accounted for 54% of all CBT in the USA, although it must be noted that this only accounted for 6% off all allogeneic HST in adults. 1

According to the Center for International Blood and Marrow Transplant Research (CIBMTR), this increase can mostly be attributed to the steady increase of the number of banked CB units which makes it easier to find two (partially) matched units for a particular patient for double CBT (dCBT). The increase in CBT is reflected by the fact that research focusing on the improvement of CBT is still active and currently there are 418 ongoing clinical trials involving CBT. 2

Despite the major contribution of CBT over the last 25 years, several problems need to be improved to make cord blood the best choice for unrelated donor HST. The most important problem in this respect is the higher chance of graft failure and delayed engraftment, which are both related to the low number of HSPC in a cord blood graft. Another problem is the relatively high cost; CBT is on average more expensive than matched unrelated bone marrow or peripheral blood stem cell (PBSC) HST. 3 The main cost drivers in this respect are laboratory costs such as tests, imaging, injections and radiation) and the number of inpatient days, both accounting for approximately 30% of the total cost. The time that the patient has to stay in hospital is directly related to delayed engraftment seen after CBT. Another aspect of cost is the high acquisition price of a cord blood unit, especially when the patient is transplanted with two cord blood units. 4

Because of these problems in recent years the increase in the number of CBT has stagnated (figure 1). A more efficient and or alternative use of banked CBs would be extremely important to reduce and compensate logistic costs while faster engraftment of a CB graft would reduce morbidity and mortality and lower the related patient treatment costs.

Figure 1: Number of allogeneic transplantations per donor source in centers that are registered with the CIBMTR for patients older than 20 years (A) and younger than 20 years (B). Figure adapted from the CIBMTR summary slides 2014 5 (UCB: umbilical cord blood, BM: bone marrow, PB: mobilized peripheral blood stem cells, URD: unrelated donor)
This thesis focuses firstly on possibilities to improve the engraftment after CBT by the co-
transplantation of mesenchymal stem cells (MSC) (chapter 1), the in vitro expansion of CB
(chapters 2&3) or a combination of both expansion and MSC co-transplantation
(chapter 4). Secondly, by investigating the use of CB for other cellular therapies without
compromising the potential for HST a reduction of the cost for a CB product is (chapter
5).

Co-transplantation of mesenchymal stem cells and CB to enhance the engraftment
of HSC: practical considerations
For large-scale application of co-transplantation of MSC for HST, there is a need for an, of
the shelf, low cost source of MSC. In this thesis we studied the engraftment enhancing
effect three different sources of MSC, fetal lung (chapter 4), adult bone marrow and the
Wharton's Jelly of the umbilical cord (chapter 1). We initially used fetal lung MSC since
this source of MSC was previously used and shown to significantly improve the
engraftment of CB CD34+ cells. However, these MSC are harvested from aborted fetal
tissues and besides ethical opposition to the use of such tissues, it is doubtful that the
limited availability of this source is compatible with clinical applications on a large scale.
In chapter 1 we show that the engraftment enhancing capacity of MSC harvested from the
Wharton's Jelly (WJ) of the umbilical cord is similar to that of BM derived MSC. WJ in this
respect is a more attractive source for the clinical application of MSC for several reasons:
1) The umbilical cord is considered to be waste material and is therefore practical free of
ethical constraints. In contrast, MSC, harvest from the bone marrow and adipose tissue as
other possible sources both require invasive and more or less painful procedures, i.e.
aspiration of BM from the pelvis and liposuction respectively. Most ethical review boards
are therefore reluctant to harvest MSC from these sources from non-related donors on a
large scale. 2) If the umbilical cord containing the WJ is collected concurrently with the CB,
the costs of HLA typing and infection screening of the donor, but also part of the collection
logistics can be shared between both products, logically resulting in a reduction of the cost
of both CB and a WJ MSC product. 3) Finally, WJ MSC are of fetal origin and therefore have
a lower chance of viral transmission such as Epstein Barr virus and cytomegalovirus. A
disadvantage, however, is that cord collections are far from sterile while BM or adipose
aspiration can be done in a closed aseptic manner. Indeed, when we developed the
protocol for the isolation of WJ MSC (chapter 1), we were initially confronted with a
variety of infections ranging from opportunistic bacteria to fungal infections. These
infections could be virtually prevented by implementing a stringent disinfection protocol
in which both ends of the umbilical cord are clamped to prevent influx of collection
medium into the cord, followed by sterilization of the outside of the cord with first iodine
and then 70% ethanol. We have chosen an 'explant' method to isolate the MSC instead of
digestion of the cord tissue with enzymes because it is less laborious and cost intensive
than digestion of the tissue with enzymes. This method, despite challenges, has shown
that isolation and culture of WJ MSC can be performed with GMP compliance. Moreover,
since the engraftment enhancing effect of WJ MSC is similar to that of BM MSC WJ MSC
could be a suitable alternative for BM MSC and probably more convenient for large scale
clinical application.

Co-transplantation of mesenchymal stem cells and CB to enhance the engraftment
of HSC: scientific considerations
A large conceptual problem for all clinical applications of MSC, including the enhancement
of HST engraftment, is the variability of the MSC between cultures. Although all harvested
and cultured cells fulfill the minimal criteria defining them as MSC, these products of the
cultures comprise a heterogeneous collection of cells, showing large variations with respect to more detailed immuno-phenotypes, proliferative capacity, differentiation capacity and the ability to secrete cytokines. Moreover, with still inconclusive knowledge on the mechanism(s) by which MSC exert their effects, we can only expect to find associations between general in vitro tests and the actual in vivo results. These associations between the in vitro capacities on the one hand and the in vivo effects on the other hand however, would be of great importance to develop in vitro quality control tests as release criteria for an effective therapeutic functionality. In chapters 1 and 4 we therefore analyzed different in vitro characteristics of MSC to see whether these characteristics could be correlated to the engraftment enhancing effect of MSC.

In theory, the engraftment enhancing effect of MSC can be achieved via 1) local mechanisms, e.g. repair of the stromal tissues in the BM or secretion of factors that assist in proliferation and/or differentiation of the HSC in the BM, or 2) systemic mechanisms, e.g. through an immunological effect on or assistance in the homing of the HSC to the BM. The BM niche is likely to be the key target of MSC therapy. This niche consists of stromal cells such as pericytes, osteoblasts, osteoclasts and adipocytes that are important for the maintenance and proliferation of HSC. This tissue is damaged by chemotherapy or irradiation of the recipient of HST and repair of the stromal components of marrow could be established by the homing of the MSC to the BM and their subsequent differentiation. Interestingly, analysis of the in vitro capacity to differentiate into osteoblast, adipocytes and chondrocytes (chapter 1) showed that WJ MSC are limited and variable in this respect compared to BM MSC. Since MSC from both sources were equally potent in enhancing the engraftment of CB CD34+ cells, it is unlikely that differentiation into stromal tissues of the recipient is responsible for the engraftment enhancing effect in our model. Moreover, and of considerable importance, homing of intravenously injected MSC to the BM has so far not been convincingly shown.

Another mechanism for the engraftment enhancement effect could be related to the immuno-modulatory capacity of MSC. The in vitro experiments in chapter 1 show that the capacity of MSC to suppress an immunological response is similar between MSC derived from WJ or BM. Notwithstanding this possibility, the 3 fold enhancement mediated by MSCs of both sources is not likely due to their immune modulating capacities since transplantation was performed with purified stem cells in immune deficient NOD SCID mice and our model was therefore devoid of immunologically active cells.

Finally, the engraftment enhancing effect of MSC could be attributed to the ability of MSC to assist HSC homing to the BM. In chapter 4 we show that MSC can improve the migration of non expanded CD34+ cells in transwell plates with an SDF-1α gradient. SDF-1α is main chemoattractant of the BM, and expression of its receptor CXCR4 is associated with the homing of HSC to the BM.

Interestingly, this increased migration was not related to the expression of CXCR4, the receptor for SDF-1α, by the CD34+ cells. This could be explained by the in vitro experimental conditions. The plates are coated with fibronectin, a ligand for adhesion receptors CD49d and CD49e. Earlier studies have shown that migration of CD34+ cells on fibronectin coated plates is increased compared to non-coated plates, and blocking of the adhesion receptors CD49d and CD49e diminishes this increase. Indeed, we could show that incubation of the CD34+ cells with fetal lung MSC did upregulate the expression of CD49d and CD49e, which could explain the increased migration described in chapter 5.

On the other hand, in chapter 2 CD34+ migration was not increased by BM or WJ MSC despite a similar upregulation in CD49d and CD49e as was observed for fetal lung MSC.
These inconsistent results leave doubt that enhancement of migration after incubation with BM or WJ MSC is the main cause for enhancement of in vivo engraftment.

Lastly, the number of passages of the cultured MSC may play a role. A study that looked at the long term outcomes of MSC treatment for GvHD and hemorrhagic cystitis showed that in the GvHD patients the one year survival rate was only 21% in the patients that were transplanted with MSC that were cultured for 3 or more passages, as opposed to 75% in the patients that were treated with MSC from passage 1 or 2.\textsuperscript{17}

Currently there are seven clinical trials involving the co-transplantation of mesenchymal stem cells listed on the website of the U.S. National Institutes of Health.\textsuperscript{2} Whether these small scale studies that mostly focus on the safety of MSC co-transplantation\textsuperscript{18-23} will give definite answers on engraftment mechanisms is doubtful and more detailed and larger in vivo studies on the mechanism behind engraftment enhancement are therefore needed to conclusively elucidate this mechanism.

**Co-transplantation of mesenchymal stem cells and CB to enhance the engraftment of HSC: safety concerns**

There are several safety concerns with respect to the clinical application of MSC.\textsuperscript{24} In order to obtain a sufficient number of MSC for transplantation, primary MSC are cultured for several passages. During this culture it is possible that the cells acquire DNA mutations and chromosomal aberrations which can lead to malignant transformation of the cells after transplantation. Fortunately, so far such malignant transformations have not been observed in vivo or in vitro.\textsuperscript{25} Another concern is ectopic tissue formation of the transplanted cells, but a study that analyzed tissue samples from 31 patients that were infused with MSC found no signs of this formation. Furthermore, the detection of DNA derived from the MSC donor correlated negatively with the time after infusion, i.e. the detectable levels of DNA coming from the MSC decreased with time, showing that the transplanted MSC gradually disappear.\textsuperscript{26}

Another safety concern associated with the co-transplantation of MSC and HSC apply to possibly unwanted immunomodulatory capacities of the MSC. Although MSC can, next to their engraftment enhancing effect, prevent GvHD, their immunomodulatory capacity can also be detrimental for the outcome of transplantation. In this respect, co-transplanted MSC might theoretically suppress the graft versus leukemia (GvL) effect and consequently increase the chance of relapse. A pilot study from 2008 showed that in a group of patients that was co-transplanted with MSC 6 out of 10 patients relapsed compared to only 3 out of 15 patients in the control group.\textsuperscript{27} However, another study that compared historical controls with patients that were co-transplanted with MSC did not find such an increase in relapse incidence, but did find a MSC associated reduction in GvHD.\textsuperscript{28}

Finally, the immunosuppressive effect of MSC could lead to a higher infection rate in patients that are co-transplanted with MSC. One of the mechanisms by which MSC suppress immune responses is the induction of indoleamine 2,3-dioxygenase (IDO), which catalyzes the conversion from tryptophan to kynurenine.\textsuperscript{29} Kynurenine decreases T-cell cytotoxicity, which can make the patient more vulnerable to infections such as CMV and EBV and opportunistic fungal infections. The previously mentioned study on the long term follow up of MSC treated patients found a high incidence of fungal infections in patients with acute steroid resistant GvHD that responded well to the therapy.\textsuperscript{17} The long term effect of immune suppression by MSC also seems to play a role in the survival rate of these patients as they also show a high mortality rate due to infections two years after treatment.

Notwithstanding the so far excellent safety record of MSC treatment, larger randomized studies with longer follow up are needed to elucidate not only how MSCs exert their
beneficial effects in vivo but also, vice versa, if they can induce relapse, infection or other longer term unwanted side effects. These studies should take the conditioning regime, the specific disease and the GvHD course and treatment as parameters into account.

Clinical application of CB expansion with TPO: practical considerations
As mentioned in the introduction of this thesis, the clinical application of the transplantation of TPO expanded cells would be most suitable for patients undergoing unexpanded CB HST that are refractory to platelet transfusions. Although we show in chapter 2 that the engrafted TPO expanded cells exhibit the potential for long term engraftment, recovery of CD45+ cells in the blood is delayed. Furthermore, the long term engraftment capacity is decreased as compared to non expanded CB cells and transplantation of a single graft of TPO expanded CD34+ cells is therefore not advisable. Transplantation of TPO expanded cells in combination with a non expanded unit to safeguard long term engraftment therefore seems to be the best alternative. Important in this respect is that the TPO expanded cells responsible for short term platelet recovery did not reduce or affect the long-term engraftment of non-expanded CD34+ cells nor influence the platelet recovery of these cells (chapter 2).
For further translation of the in vivo experiments with immune deficient mice into clinical use, we still need to monitor the functionality of platelets derived from the TPO expanded cells that have engrafted; platelets derived from non-expanded CD34+ cells that engrafted in NOD SCID mice have shown this functionality in in vitro assays 31, GMP compliant upscaling of our expansion protocol to a therapeutic transplant size would be the next step towards clinical implementation. What is already convenient of the current protocol is the use of the clinical grade Nplate (romiplostim), an analog of TPO, which is registered to treat thrombocytopenic patients 32 and the fact that the expansion medium does not contain animal components such as fetal bovine serum.

Combining TPO expansion and the co-transplantation of MSC for the improvement of CB transplantation.
Transplantation of expanded cells and the co-transplantation of MSC both seem potent strategies for the enhancement of CB engraftment. Combining these strategies could be a way to synergistically improve the outcome of CB transplantation. In chapter 4 we report on TPO- expanded CB CD34+ cells, shown to accelerate platelet recovery in the peripheral blood (PB) 33-36 with co-transplantation of MSC, shown to improve bone marrow engraftment. While both strategies themselves provided the benefits of each of the separate strategies, combining both methods did not yield any synergistic effect and even led to non engraftment events. Graft failure of CBT is believed to be caused by the low number of stem cells and/or HLA disparity between the donor and the the recipient. Both these mechanisms however are not likely to play a role in the non-engraftment events we observed in our in vivo experiments since identical grafts (and cell numbers) without MSC were able to reconstitute the bone marrow and immune cells are lacking in our model. We excluded that the non engraftment events in chapter 4 might be due to the high amounts of infused cells when the TPO expanded cells and MSC were combined and possibly concomitant lung entrapment of hematopoietic stem cells, since transplantation of the MSC four hours after the transplantation of the TPO expanded cells still resulted in a mouse without engraftment. Without any synergistic effect of the co-transplantation of MSC and TPO expanded cells on either platelet recovery or engraftment we feel that this safety aspect does not make this combined approach a viable option for further implementation.
Expansion of long-term engraftment capacity of CB HSC in one of two transplanted cords: general considerations

Since several expansion protocols are known to increase the graft size to cell numbers that should ensure successful engraftment,\textsuperscript{37-39} it is still considered to be a risk to transplant only one expanded unit into an adult patient. So far all clinical trials using expanded cells have therefore combined the expanded graft with a non expanded graft\textsuperscript{37-40} to ensure the long term engraftment capacity of the transplant. Expansion of HSC inevitably leads to a certain degree of differentiation of the long term repopulating stem cell pool whereby the immaturity and self renewal capacity is lost leading to reduction in long term repopulation capacity.

Preservation of the long term repopulating capacity cannot be assessed properly with in vitro models while animal models also have intrinsic limitations. Clinical studies that have used expanded cells in combination with a unit of non expanded cells, showed that long term engraftment is usually skewed towards or eventually totally dependent on the non-expanded unit\textsuperscript{38,40}. However, because of this double transplant approach it remains difficult to assess whether expanded cells indeed lose their long term engraftment ability or that non-expanded cells eventually out-compete the expanded cells in this respect. In light of this possibility, in chapter 2 we specifically studied graft dependent blood recovery and engraftment in double graft transplantations of a TPO expanded and a non expanded CB graft in NOD SCID mice. By the use of HLA allele specific antibodies, the progeny of the different grafts could be distinguished in the blood, BM and spleen. Although the PB of the mice contained platelets that originated from the TPO expanded graft up to 20 weeks after transplantation, thus showing that TPO expansion does not deplete the capacity of the CB cells to differentiate into megakaryocytes and platelets, the chimerism of the cells in the blood, BM and spleen at 20 weeks was skewed towards the non expanded graft. This suggests that long term engraftment of the TPO expanded cells occurs but was less efficient than that of the non-expanded cells.

Two mechanisms by which the non-expanded unit becomes dominant should be considered. So far, by looking at clinical experience with double CB transplantation of two non expanded units, immunological processes and the viability and cell number of the individual units seem to be the most important factors in the establishment of dominance. The number of specific immune competent T cells such as CD3 (CD4+CCR7+) cells in either graft seems to be associated to the establishment of dominance of that particular CB unit.\textsuperscript{41-43} The remaining number of these types of cells in an expanded graft in this respect will depend on the type of/nature of expansion protocol. If the cells are expanded for the mononuclear fraction of the cord blood unit, the immune competent cells are likely to be affected by the culture conditions while in isolated and expanded CD133+ and CD34+ cells the immune competent cells are completely removed.

In the latter approach, the non expanded cells will clearly have the immunological advantage in a graft vs graft response leading to eventual dominance of this unit. In our in vivo model that was devoid of immune competent cells, the observed dominance of the non expanded cells should be attributable to another mechanism and possibly dependent on the number of (long term repopulating) stem cells that was higher in the non-expanded units.

The immune competence of grafts is not only important for engraftment and dominance in case of double transplant strategies, it is even more important if one realizes that the (minor) HLA mismatch between the graft and the patient is critical for the GvL effect. GvL is essential to prevent disease relapse and in essence required for total donor chimerism and eventual complete disease remission.\textsuperscript{44} Expanded and non-expanded CB contain only low numbers of effective immunocompetent T cells but nevertheless show a similar
relapse rate as seen with matched unrelated donor grafts. However, in the case of relapse, additional blood from a CBT donor cannot be harvested for donor lymphocyte infusions (DLI). 45 A solution for this might be the expansion of T cells from the CD34 negative fraction after isolation of the stem cells prior to expansion. 46

As mentioned above the main challenge is the expansion of the true long term repopulating hematopoietic stem. 47 The CD34+ cell population in CB is a heterogeneous population containing only a limited number of immature progenitors (long repopulating HSC) with the ability of complete self renewal and mostly consists of more mature progenitors (short term repopulating HSC) and committed myeloid or lymphoid progenitors. 48,49

Earlier expansion protocols used a combination of cytokines that led to a high degree of differentiation and which coincides with the maturation of the most immature cells and thus loss of the long term repopulating capacity of the graft. Although a single long term repopulating cell is capable of eventual complete reconstitution of the bone marrow, 50 the speed to attain this is most critical for limiting morbidity and the mortality of the patients. More recent expansion protocols focus on the preservation of the true long term repopulating stem cell (LT HSC) by adding small molecules to the expansion medium. An example of one of these small molecules is Stemregenin 1 (SR1), an arylhydrocarbon receptor (AHR) antagonist. AHR is an important regulator of hematopoietic stem cells 51 and culture with SR1 has shown to increase the number of LT HSC in vitro and in immune deficient mouse models. 52,53 Because of the promising experimental results, a phase 1 trial has started to evaluate the clinical efficacy of expansion with SR1. 54 Other examples of small molecules that can increase the expansion of LT HSC are transcriptional such as UM171, a pyrimidoindole derivative, 55 and valproic acid, and histone deacetylase inhibitor. 56 However, as mentioned before, long term repopulation is difficult to assess in vitro or in animal models, and larger scale clinical trials are needed to investigate the value of these expansion strategies.

**CB engraftment stimulating protocols: logistic considerations.**

In chapter 3 we show that the engraftment capacity of CB CD34+ cells expanded with TPO before and after cryopreservation is similar. However, as discussed in this chapter, expansion before cryopreservation is logistically largely impractical since it is not known which cord blood unit is going to be needed for transplantation and expansion of all collected units would be an extremely costly exercise. However, expansion from a cryopreserved unit is neither an easy option since it needs to be performed in a GMP certified cell culture facility and not all transplantation centers have such a facility available. A solution for this would be to centralize the expansion of the CB unit upon request at the CB banks.

In general, the engraftment enhancing strategies for CB transplantation that were investigated in this thesis have shown their merit in in vivo models, but before these strategies can be applied clinically, several practical and scientific issues have to be addressed. The most complicating issue in this respect is that before actual use in patients becomes possible, that the manufacturing of the involved cellular products, either ex vivo culture of HSC or the culture of accessory cells such as MSC or regulatory T cells, not only require GMP grade facilities, but also highly qualified personnel and rigorous compliance to laboratory protocols. Indeed, the various manipulations needed for these cell therapies make them so called advanced therapy medicinal products (ATMP) falling under medicinal regulations and new approval for each change in the manufacturing protocol has to be applied for.
Because of all these practical and regulatory hurdles, it would probably be advisable to prepare the grafts for these still experimental cell therapies in the specialized transplantation centers or blood bank facilities that are not only equipped to process cellular products under GMP conditions but also to facilitate validated GCP grade assays including immune and engraftment monitoring. Only under such conditions fast advance of knowledge is ensured and can reverse translation of outcomes lead to optimization of these therapies.

**The use of CB for other purposes than HST**
CB also contains non-hematopoietic cells that might be useful for regenerative or immunomodulatory cell therapies. The best known examples of these cells are endothelial progenitor cells (EPC) and MSC. The number of endothelial progenitor cells and MSC in CB is however very low e.g. for 2.99±2.45 endothelial progenitor cells per 10⁸ mononuclear cells (MNC), ⁵⁷ or 0-2.3 MSC per 10⁸ MNC, ⁵⁸ and the culture of these cells has proven to be difficult. ⁵⁸ What factors influence the number of non-hematopoietic cells in CB is unknown, although the gestational age of the cord blood seems to play a role. ⁵⁹ CB that was collected after 24 to 28 weeks of gestation seems to contain more MSC, whereas CB from weeks 33-36 predominantly contains EPC.

The regenerative potential of the non hematopoietic cells from CB is, with the exception of MSC immunotherapy, mostly useful in an autologous setting since therapies such as wound repair and bone or cartilage grafting requires the transplantation of live tissue that can be rejected by the host if the cells are of an allogeneic origin. Publicly stored CB is exclusively banked for the purpose of allogeneic HST. Because only a few percent of the stored CB units is actually used for this purpose, CB banking is a costly activity. The number of privately banked CB units is almost double the number of publically banked units and virtually not used. ⁶⁰

Private CB banking initiatives successfully advertise autologous stored CB as superior treatment of (future) hematological malignancies of the child itself or one of its family members. The theoretical chance of needing the CB for the advertised purpose is only 1 in 2700 banked units which are estimated to be of potential benefit for the donor itself or its family. ⁶¹ Finding a way to utilize a single CB unit for both autologous (non-hematopoietic) and allogeneic (HSCT) purposes could have considerable advantages, as the ‘dual’ use of banked CB could be partly paid for by additional interest and financial support of the donors.

To explore this ‘dual banking’ strategy we investigated in chapter 5 if the hematopoietic cells in CB could be separated from the non-hematopoietic cells based on the expression of the pan hematopoietic marker CD45. We specifically investigated whether removal of the CD45 negative cells would affect the in vitro and in vivo hematopoietic potential of the graft. The ability to form hematopoietic colonies in culture is correlated to the platelet and neutrophil engraftment capacity of a CB unit. ⁶² Removal of the CD45-negative cells neither affected the number of hematopoietic colonies that were formed by CD45-positive cells in a standard methocult assay. In agreement the CD45- negative cells showed no hematopoietic colony forming capacity. In mixed lymphocyte cultures, the CD45- cells were not immunogenic themselves and did not influence the PBMC response to CD45-positive cells or vice versa. We therefore hypothesize that CD45-negative cell removal will not likely influence the immunological properties of the remaining graft, which is important for both the incidence of GvHD and the GvL effect. Although our NOD-SCID transplant model does not test immunologic mechanisms, co-transplantation of CD45-negative cells with CD34+ cells did not change engraftment in NOD SCID mice of CD34+ cells alone. In contrast, co-transplantation of CD45-positive with CD34+ cells clearly
showed the added engraftment enhancing potential of the CD45+ population. The possible non-hematopoietic use of the remaining CD45- cells after CD45+ immuno-separation was shown by the fact that endothelial cell colonies could be cultured from the isolated CD45- cells but not from the CD45+ cells.

Our results, although preliminary, suggest that CD45-negative cells from CB might be used separately without altering the HST capacity of the CD45-positive cells. However, it must be noted that our transplant model does not allow for the testing of immunological mechanisms. Our findings should therefore be confirmed in models that better reflect the human setting in this respect. There are several practical and logistic obstacles for this ‘dual-use’ banking initiative. Firstly, the time that cells are needed for either autologous or allogeneic use will usually not coincide. This will make pre-cryopreservation separation and separate storage more preferable with respect to the quality of the cells, but this will increase the cost of banking. Again, the problem with this strategy is that it is not known beforehand which specific cell type of which particular donor will be needed in the future and culturing and banking a variety of different cell types would increase the cost for processing and banking of the CB unit. Therefore, post cryopreservation separation and culture is more practical and likely to be the only option that can be considered. However, although endothelial progenitor cells can be cultured from cord blood that was stored for over 20 years, the culture of MSC from frozen CB is very difficult. Loss of cells by the freeze thaw procedures combined with the already low and variable number of non-hematopoietic cells in CB will therefore limit the number of successful cell cultures from cryopreserved CB.

For MSC, considered as one of the most promising cell types for cell therapies that can also be used in an allogeneic setting, it is important to realize that they can be more easily obtained from the Wharton’s Jelly of the UC itself and private CB banks have started to offer the storage of these cells. In this respect it must be noted that WJ MSC show clear aberrations from the typical MSC phenotype and its therapeutic use can therefore not be extrapolated from findings obtained with other MSC sources. In chapter 1 we found a limited and variable differentiation capacity into osteoblast, adipocytes and chondrocytes which was previously shown by earlier studies. However, and perhaps more important, WJ MSC were shown to possess important in vitro immunomodulatory and in vivo engraftment enhancing capacities that are similar to BM MSC, which are much more difficult to obtain.

In conclusion, new use of cord and cord blood cells seem possible. Adapted cord and cord blood banking strategies and manipulation strategies (most likely post cryopreservation) are however, needed for this new use and the cost for this is considerate and should, depending on the development of the progress in cellular therapies be weighed carefully. This new use not only involves creating greater HST efficacy of cord blood but also non-hematopoietic like regenerative and more specific immunomodulatory indications. Isolation of the CD45-positive cell from CB for the use in HSCT, in this regard could make the remaining CD45-negative cells a new asset for these purposes. The logistics and methodology to optimize such new therapeutic vistas need consistent bench to bed side synergies between scientists and clinicians, but also dedicated grants supporting this vision and international and multicenter collaboration.

**CB transplantation or haploidentical transplantation: the choice of the right graft source**

Despite many scientific and clinical advances, the increase in CB transplantation seems to have stagnated in the last years (Figure 1). This stagnation might be due to center specific preferences in using other unrelated donor transplants such as haploidentical grafts from
a child or parent of the patient \textsuperscript{65} or the use of mismatched (un)related donor grafts. \textsuperscript{66} Another reason is that high incidence of graft versus host disease (GvHD), which was historically a big problem with haploidentical graft transplantation \textsuperscript{67,68}, is more manageable. Besides T-cell depletion as a main preventive measure, large progress has been made in the understanding, treatment \textsuperscript{[9]} and prevention of GvHD, such as the post-transplant administration of methotrexate \textsuperscript{[10,11]}. Additionally, experimental therapies are now being investigated such as the treatment with MSC infusions both prophylactically \textsuperscript{28,69} or when first line treatment for GvHD with corticoids fails. \textsuperscript{70-75} Alternatively, co-transplantation of regulatory T cells has shown promising results in prevention of GvHD. \textsuperscript{76,77} This improved treatment and prevention of GvHD has revived the interest in haploidentical HST.

Haploidentical grafts also have other advantages over CB grafts. \textsuperscript{78-80} One of these advantages favoring haploidentical HST is the low acquisition cost. Secondly, a haploidentical donor graft is usually quickly available since nearly every patient has a haploidentical sibling, child or parent. In contrast, for CB, despite the increasing number of banked units, the variability of the cell dose in the banked units still limits the choice of sufficiently large grafts. Thirdly, the immune reconstitution after haploidentical transplantation is still faster compared to CB transplantation and reduces the risk of infection, the largest cause of non-relapse mortality, especially in the first three months after transplantation \textsuperscript{81}. A last and often mentioned advantage of non-CB grafts is the possibility of post-transplant donor lymphocyte infusions (DLI) to prevent or treat relapse. Whether this applies to haploidentical grafts is however debatable since haploidentical DLI –although possible- carries again a very high risk of GVHD and is therefore very rarely applied. Conversely the strong T cell depletion needed to control GVHD in the case of haploidentical GVHD is offset by a higher relapse rate, and T cell replete grafts \textsuperscript{82} or post-transplant DLI \textsuperscript{83} in these patients again increases the risk of GvHD. Hence, development of more practical relapse treatment solutions for both CB and haploidentical transplantations is therefore needed and crucial for choice of the most suitable graft source. In this respect, DLI from CB in the near future is likely to become possible by – although costly - expansion of T cells from the CD34 negative fraction of the graft. \textsuperscript{46}

Finally, it is good to realize that most comparisons between different graft sources are based on different studies performed in separate centers with retrospective outcome analyses. Moreover, variability in patient groups and diseases treated in these studies make these comparisons even more complicated. While certain graft sources might be better in the treatment of a certain group of patients or of a certain disease, the experience of a center with a certain transplant platform/approach is probably one of the most critical determinants to optimize the disease and patient related outcome. Prospective randomized and multicenter studies not only covering morbidity and mortality but also treatment costs and quality of life issues are therefore needed to correctly evaluate the pros and cons of each graft source. \textsuperscript{84,85} The continuing research on the enhancement of CB transplantation, such as HSC and lymphocyte expansion, however, is accelerating and could improve the outcome for CB recipients in such a way that this might shift the balance for graft choice towards CB.
References

16. Voermans C, Gerritsen WR, van dem Borne AE, van der Schoot CE. Increased migration of cord blood-derived CD34+ cells, as compared to bone marrow and mobilized peripheral blood CD34+ cells across uncoated or fibronectin-coated filters. Experimental hematology 1999;27: 1806-14.
51. Singh KP, Casado FL, Opanashuk LA, Gasiewicz TA. The aryl hydrocarbon receptor has a normal function in the regulation of hematopoietic and other stem/progenitor cell populations. Biochemical pharmacology 2009;77: 577-87.
76. Di Ianni M, Falzetti F, Carotti A et al. Tregs prevent GVHD and promote immune reconstitution in HLA-