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**Title:** Different strategies to improve the use of the umbilical cord and cord blood for hematopoietic and other regenerative cell therapies  
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Summary
Umbilical cord blood (CB) is an alternative for mobilized peripheral blood stem cell or bone marrow grafts when a patient needs allogeneic hematopoietic stem cell transplantation (HST). However, CB HST is associated with increased incidence of graft failure and delayed engraftment and immune reconstitution compared to other graft sources, which is caused by the relatively low numbers of stem cells in a cord blood unit. There are a number of strategies that can be used to increase the number of stem cells in a cord blood graft, including double CB transplantation, ex vivo expansion of CB and the co-transplantation of accessory cells such as mesenchymal stem cells (MSC). For this thesis we investigated different aspects of the application of these strategies. The co-transplantation of MSC has shown promising results in animal models. It enhances the engraftment of the stem cells leading to higher numbers of cells in the hematopoietic organs of the recipient. The majority of MSC used in the clinic are derived from the bone marrow of healthy donors. The harvest of these MSC is a costly procedure and involves a certain risk for the donor, and is therefore ethically constrained.

We compared in vitro characteristics and the engraftment enhancing capacity of BM MSC with MSC from the Wharton jelly of the umbilical cord (WJ MSC). These MSC have several advantages over BM MSC such as a low collection costs and a relatively low chance of transmissible infections. Furthermore, being a waste product, WJ MSC are free of ethical constraints. Although less capable of differentiating into mesodermal lineages, WJ MSC have a similar capacity to inhibit immunological responses in vitro and to enhance the engraftment of CB CD34+ cells in vivo. WJ MSC would therefore be a good alternative for the use of BM MSC from healthy donors.

Ex vivo expansion of CB has successfully been applied in several phase I/II clinical trials. Our lab has previously developed a protocol for the expansion of CB CD34+ cells, using only TPO, which can improve platelet recovery in the peripheral blood. Expanded cells with this capacity would be especially helpful for patients that are refractory to platelet transfusions, since these patient have an increased risk of dying from hemorrhage after HST. For clinical application of these expanded cells, co-transplantation of non expanded cells is necessary to safeguard the long term reconstitution of all blood cells. We investigated the transplantation of expanded cells and non expanded CB cells and did not find any difference with respect to recovery of CD45+ cells in the peripheral blood of the mice or BM and spleen engraftment when compared to the transplantation of unmanipulated cells from two CB units. Platelet recovery was significantly improved in the first weeks after transplantation, which suggests that this strategy would be favorable over the transplantation of two unmanipulated units. We further investigated if the order of cryopreservation and expansion, i.e. before or after the banking of the CB unit, would influence the outcome of transplantation. Although the number of cells that are formed after expansion are higher when the CB cells are expanded before the cells are cryopreserved, we did not find any significant differences in the engraftment capacity compared to cells that were expanded after cryopreservation of the CB cells. The latter even proved to be more efficient in the recovery of platelets in the peripheral blood of the mice. This suggests that our expansion protocol can be applied to all CB units that are currently stored in CB banks.

Lastly we tried to combine the co-transplantation of MSC with the transplantation of TPO expanded cells. Each strategy by itself can either improve overall engraftment or early platelet recovery in the peripheral blood. A combination of both strategies could therefore further improve the outcome of CB transplantation. However, we found that, although each single strategy improved the outcome of transplantation in the way we expected, the combination of strategies did neither further improve early platelet recovery nor engraftment. Furthermore, we encountered events of non engraftment in several mice,
which suggest that the combination of MSC and expanded cells can lead to unwanted adverse events. We could not determine the cause of these adverse events and therefore concluded that this strategy cannot be translated into a clinical protocol until the cause leading to these events is elucidated.

**Recommendations**

WJ MSC are a promising source of MSC for clinical application. We therefore recommend that the protocol for the collection of the umbilical cord and processing of the cells should be translated into a GMP grade protocol.

Transplantation of TPO expanded cells in combination with unmanipulated cells seems to be safe with respect to the early recovery of platelets and the long repopulation of the BM. The expansion protocol could therefore be translated into a GMP grade protocol and simultaneously scaled up from culturing small quantities of cells to whole CB units. A phase I clinical trial with TPO expanded cells in combination with unmanipulated CB cells would be an option if there is demand from clinicians that have a sufficient number of patients that would benefit from the transplantation of these cells.