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# Therapeutic Perspectives for the Clinical Application of Umbilical Cord Hematopoietic and Mesenchymal Stem Cells: Overcoming Complications Arising After Allogeneic Hematopoietic Stem Cell Transplantation

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## Abstract

This review focuses on the therapeutic features of umbilical cord blood (UCB) cells as a source for allogeneic hematopoietic stem cell transplantation (aHSCT) in adult and child populations to treat malignant and nonmalignant hematologic diseases, genetic disorders, or pathologies of the immune system, when standard treatment (e.g., chemotherapy) is not effective or clinically contraindicated. In this article, we summarize the immunological properties and the advantages and disadvantages of using UCB stem cells and discuss a variety of treatment outcomes using different sources of stem cells from different donors both in adults and pediatric population. We also highlight the critical properties (total nucleated cell dose depending on HLA compatibility) of UCB cells that reach better survival rates, reveal the advantages of double versus single cord blood unit transplantation, and present recommendations from the most recent studies. Moreover, we summarize the mechanism of action and potential benefit of mesenchymal umbilical cord cells and indicate the most common posttransplantation complications.

## Keywords

Hematologic diseases · Hematopoietic stem cell · Mesenchymal stem cell · Stem cell transplantation · Umbilical cord

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## Abbreviations

| | |
|---|---|
| ABMI | Autologous bone marrow cell infusion |
| aGvHD | Acute graft-versus-host disease |
| aHSCT | Allogeneic hematopoietic stem cell transplantation |
| ALL | Acute lymphoblastic leukemia |
| AML | Acute myeloid leukemia |
| ATG | Anti-thymoglobulin |
| BM | Bone marrow |
| BMMSC | Bone marrow mesenchymal stem cells |
| BMT | Bone marrow transplantation |
| CAR | Chimeric antigen receptor |
| cGvHD | Chronic GvHD |
| CMV | Cytomegalovirus |
| DCs | Dendritic cells |
| DFS | Disease-free survival |
| DL-1 | Delta-like ligand 1 |
| DLI | Donor lymphocytes infusion |
| dUCBT | Double UCBT |
| GM-CSF | Granulocyte-macrophage colony-stimulating factor |
| GVHD | Graft-versus-host disease |
| GVL | Graft versus leukemia |
| HLA | Human leukocyte antigen |
| HSC | Hematopoietic stem cells |
| IL-3 | Interleukin-3 |
| IPSC | Induced progenitor cells |
| LC | Liver cirrhosis |
| LFS | Leukemia-free survival |
| MSC | Mesenchymal stem cells |
| MSD | Matched sibling donor |
| Mtx | Methotrexate |
| MUD | Matched unrelated donor |
| NF-kB | Nuclear factor kappa B |
| PB | Peripheral blood |
| PBSC | Peripheral blood stem cells |
| PGE2 | Prostaglandin E2 |
| SC | Stem cells |
| SCID | Severe combined immunodeficient mice |
| SR-1 | Stem Regenin-1 |
| sUCBT | Single UCB |
| TNC | Total nucleated cells |
| TRM | Transplant related mortality |
| UC | Umbilical cord |
| UCB | Umbilical cord blood |
| UCBT | Umbilical cord blood transplantation |
| UM171 | HSC agonist pyrimido-indole derivative |
| VST | Virus-specific T cells |

## 1 Introduction

Allogeneic hematopoietic stem cell transplantation (aHSCT) is a procedure for treating malignant and nonmalignant hematologic diseases, genetic disorders, or pathologies of the immune system when standard treatment (e.g., chemotherapy) is not effective or clinically contraindicated. The usual sources of stem cells for aHSCT are bone marrow (BM) or peripheral blood (PB) (Anasetti et al. 2012). Umbilical cord blood (UCB) cells remain an alternative source, but the advantages and disadvantages of these cells are still highly investigated and discussed. A low risk of graft-versus-host disease (GVHD) and the opportunity to use unrelated transplants suggest that UCB is a desirable candidate for transplantation (He et al. 2005).

The first successful UCB transplantation was performed in 1988 in a child with Fanconi anemia, and since then, it is estimated that over 40,000 UCB transplants have been performed both for adults and children (Gluckman et al. 1989; Mayani et al. 2020). The first cord blood bank was established in 1993, and since that time, five million cord blood units have been banked all over the world (Gupta and Wagner 2020; Dessels et al. 2018). Approximately 800,000 of these are stored in public banks, while others are kept in private banks (Dessels et al. 2018).

This review article mainly focuses on the importance of UCB stem cells as an alternative source for aHSCT and additional therapeutic properties that may facilitate the rate of successful outcomes of aHSCT.

## 2 Umbilical Cord Stem Cells

The umbilical cord is a narrow tube that connects the developing embryo to the placenta. The outer membrane of the umbilical cord is rich in stem cells (SC) (Anasetti et al. 2012). Mesenchymal stem cells (MSCs) isolated from the arteries, vein, cord lining, and Wharton's jelly have been shown to exhibit phenotypic plasticity, adherence, multipotency, and the capacity to differentiate into many cell types such as osteoblasts, adipocytes, chondrocytes, hepatocytes, and neural and cardiac cells (Molecule boosts numbers of stem cells in umbilical cord blood 2014). While Wharton's jelly has a lower density of MSCs, the large amount of this tissue allows a high number of proliferative cells to be isolated from it. Wharton's jelly-derived MSCs have a high proliferation potency and do not produce teratogenic or carcinogenic effects after subsequent transplantation (Malgieri et al. 2010). Human umbilical cord perivascular cells are almost identical to Wharton's jelly-derived MSCs (Arno et al. 2011).

The cell density of UCB hematopoietic precursors is similar to that of the bone marrow. The main differences are the higher amounts of erythroid and immature granulocyte-monocyte precursors found in the umbilical cord. The UCB contains a mixture of primitive cell precursors that form abundant colonies of rapidly proliferating hematopoietic cells and cells that cannot form colonies in semisolid cultures but can colonize after a few weeks in Dexter-type long-term cultures (Mayani and Lansdorp 1998).

## 3 Features of Umbilical Cord Blood Hematopoietic Stem Cells

UCB is a rich source of hematopoietic stem cells (HSC) and progenitor cells. UCB cells are distinguished by the higher levels of CD34+ antigen and longer telomeres than found in adult HSC (Mayani et al. 2020; Mayani 2010). In addition, they divide more rapidly as they exit the G0/G1 phase of the cell cycle more quickly (Mayani et al. 2020; Mayani 2010). Higher self-renewal capacity is determined by the overexpression of certain transcription factors (e.g., NF-kB) (Mayani et al. 2020; Mayani 2010). Moreover, UCB cells have the ability to produce cytokines, such as granulocyte-macrophage colony-stimulating factor (GM-CSF) or interleukin-3 (IL-3) (Mayani et al. 2020; Mayani 2010). Regarding these features, UCB cells can be expanded in vitro in experimental models that in the future may become available in everyday practice.

In contrast to the bone marrow and adult peripheral blood, umbilical cord blood has several potential benefits: the broad availability of cells and less stringent requirements for donor and recipient HLA compatibility reduce the waiting time to receive the transplant. Additionally, the immaturity of UCB cells is related to lower immunogenicity. UCB-derived dendritic cells have lower antigen-presenting activity, which results in lower chances of graft-versus-host disease (GVHD) (Beksac 2016).

The benefits, challenges, limitations, and problems associated with the use of UCB HSCs are summarized in Fig. 1.

## 4 Therapeutic Peculiarities of UCB Hematopoietic Stem Cells

### 4.1 Umbilical Cord Blood Stem Cells (UCBSC) Versus Bone Marrow or Peripheral Blood Stem Cells

UCB can be collected noninvasively and ensures prompt availability of stem cells. In countries where public cord blood banks are available, the number of potential donors is higher than bone marrow donors (Malgieri et al. 2010). Procedures for obtaining UCB SC pose no ethical or technical restrictions and are painless (Malgieri et al. 2010). Neither it interferes with the delayed umbilical cord clamping which is defined as 30–60 s after birth (Delayed umbilical cord clamping after birth 2020) and is recommended for vigorous term and preterm infants. Delayed

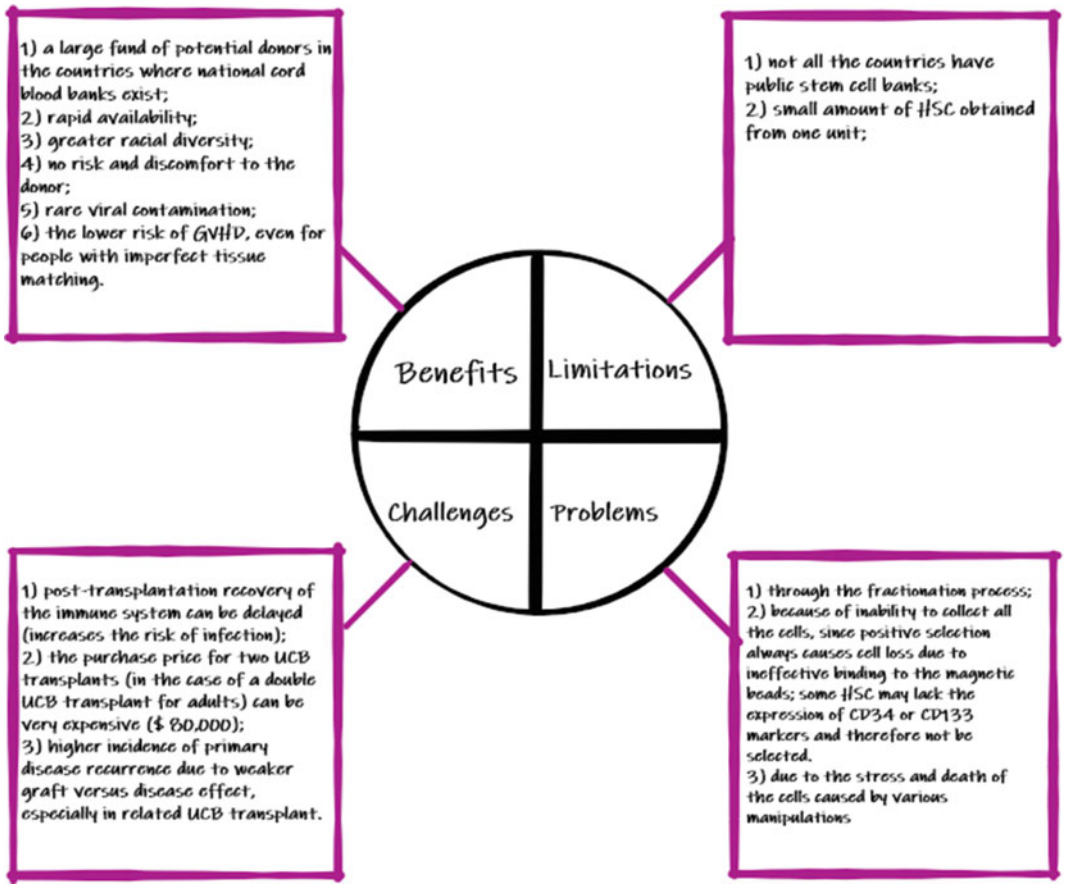


**Fig. 1** Benefits, challenges, limitations, and problems related to the use of UCB HSCs (Beksac 2016; Expansion of human cord blood hematopoietic stem cells for transplantation 2010)

cord clamping has many advantages in the early postnatal period as well as in the later childhood (e.g., lower risk of anemia, favorable developmental course). However, there are no evidences that clamping later than 3 min could be more beneficial than 30–60 s (Preterm labour and birth 2020). Thus, after labor, it is possible to reconcile both: delayed umbilical cord clamping and collection of stem cell. The cord blood can be stored in advance. In contrast, bone marrow cells have to be collected directly before transplantation. As a result, there is a potential risk of last-minute consent refusal (Malgieri et al. 2010).

Owing to all the mentioned properties, the waiting time for the transplantation procedure can be shortened by up to 2 weeks compared with 3–4 months for BMT or PBSCT (Gupta and Wagner 2020).

Besides easier and shorter procedures for obtaining stem cells, the most significant advantage of UCB is the possibility of avoiding the most serious complications after aHSCT. Firstly, there is a reduced likelihood of transmitted infections, especially human cytomegalovirus (CMV) (Algeri et al. 2020). CMV reactivation after aHSCT is one of the main clinical challenges to overcome as it often may become lethal during active immunosuppressive therapy, a treatment which is necessary to avoid or to treat acute graft-versus-host disease (aGvHD). Secondly, aGvHD is the main reason for morbidity and mortality after aHSCT. The success of transplantation usually depends on the management of aGvHD. There are many studies analyzing the probability of aGvHD using different sources of stem cells. Most of them state that UCB

transplants have a lower likelihood of aGvHD (Oran and Shpall 2012; Locatelli et al. 2014) since UCB cells are more naive and less alloreactive than adult-type HSC(Algeri et al. 2020). However, the probability of this disease depends on the HLA matching, as one or two incompatibilities increase the rate of aGvHD and chronic GvHD (cGvHD) (Eapen et al. 2017; Gabelli et al. 2020; Rubinstein et al. 1998). Double UCBT (dUCBT) is also associated with greater risk of GvHD (Wang et al. 2019).

The main negative aspects of UCB limiting its use are the delay in engraftment and immunologic recovery. Later after administration, immune T cell reconstitution poses a higher risk of opportunistic infections during the first 3 months after transplantation (Szabolcs and Niedzwiecki 2007; Jacobson et al. 2012). It has been noted that the use of anti-thymoglobulin (ATG) to prevent aGvHD has a negative effect on early CD4+ T lymphocyte recovery (Dahlberg and Milano 2017). In order to keep the balance between controlled aGvHD and immune reconstitution, it is now advised to keep sufficiently high ATG exposure in peritransplantation period of UCB and low exposure during posttransplantation period (de Koning et al. 2017).

To achieve the best outcome of UCB transplantation, the UCBT unit should contain 2,5–3 $\times$ $10^7$ total nucleated cells (TNC)/recipient's kg before thawing (Gluckman et al. 2004; Rocha et al. 2009; Hough et al. 2016). However, there are several strategies being developed to overcome this obstacle: UCB expansion ex vivo, improving homing in vivo, selection of optimal UCBT unit, and enhancement of immune recovery (Mayani et al. 2020; Ballen et al. 2013; Dahlberg and Milano 2017).

## 4.2 Umbilical Cord Blood Transplantation (UCBT) Versus Haploidentical Transplantation (HIT)

Usually, UCB cells are selected as a source for aHSCT when there is no matched related (sibling) donor (MSD) or matched unrelated donor (MUD) available. Only one in four patients may have a MSD for the aHSCT (Algeri et al. 2020; Copelan 2006; Rocha and Locatelli 2008). The possibility of finding a suitable MUD ranges between 16% and 75% depending on the recipient's ethnicity and race(Gragert et al. 2014) and usually is extremely low for racial minorities or mixed ethnicity people (Barker et al. 2010). Thus, UCB transplantation could be the only option as cord stem cells require less HLA compatibility.

Another alternative to UCBT during the last 20 years arises from haploidentical donor selection (Algeri et al. 2020; Passweg et al. 2016). The priority between these two procedures is being intensively discussed. The recent meta-analysis has revealed that UCB and haploidentical transplantation (HIT) are equally effective options for transplantation both in adults and in the pediatric population. But emphasis was made on UCB in children, as UCB ensures lower probability of aGvHD (Locatelli et al. 2014). However, considering that mostly pediatric population was transplanted due to acute myeloid leukemia (AML), UCBT was superior to HIT (Locatelli et al. 2014). It is speculated that UCB transplants could be a second choice after transplantation of material from sibling donors in children.

## 4.3 UCB Transplantation for Adolescents and Adults

To overcome the small number of cells, and to perform transplantation for adolescents and adults successfully, there has emerged an idea of double unit umbilical cord blood transplantation (dUCBT). However, the latest data has revealed that dUCBT had no advantage to single UCBT (sUCBT) when an adequate amount of TNC (>2,5 $\times$ $10^7$/kg) is transfused (Wagner et al. 2014; Michel et al. 2016; Brunstein et al. 2010). dUCBT recipients usually experience higher rates of GvHD (Rocha and Locatelli 2008), but overall survival (OS) and disease-free survival (DFS), as well as time to engraftment, were the same between sUCBT and dUCBT groups (Brunstein et al. 2010; Baron et al. 2017). Interestingly, in almost all the cases of dUCBT, only one unit

dominated owing to competition between two units and the rejection of one of them (Ramirez et al. 2012). However, regarding transplantation with positive MRD before the procedure, dUCBT may have a positive effect – lower relapse rate in comparison with sUCBT (Balligand et al. 2019). This phenomenon may be explained by higher alloreactivity and higher graft versus leukemia (GVL) effect of dUCB (Milano et al. 2016). Interestingly, it is speculated that dUCBT could be a better option than BMT for patients with positive pretransplant MRD (Milano et al. 2016). However, the limitation of this alloreactivity is higher rates of GvHD. It is worth mentioning that the GVL effect depends on the conditioning regimens (e.g., ATG), as ATG may suppress it (Balligand et al. 2019).

When the UCBT unit does not contain enough TNC for adult transplantation, another option can be chosen – the transfusion of UCB with mobilized PB derived CD34+ from a haploidentical donor (Mayani et al. 2020). In this scenario, usually early haploidentical engraftment occurs, and then it is substituted by the durable UCB engraftment (Mayani et al. 2020). This procedure has already shown early hematologic recovery, low incidence of GvHD, and durable remission (Eapen et al. 2007, 2011).

Currently, for adolescent and adult populations, UCB should be considered when there is no MSD or MUD and the transplant is needed urgently (Mayani et al. 2020).

## 5 Options to Increase UCBC Dosing

Cell dose (e.g., $> 3 \times 10^7$ TNC/kg) has an impact on better engraftment for higher than 5/6 HLA matched grafts; however, it has no effect on less than 4/6 HLA grafts (Eapen et al. 2007). The matching criteria for UCBT were based on low/intermediate resolution of HLA A and HLA B and high resolution of HLA DRB1. Since 2011, the importance of HLA C has been described (Beksac 2016; Eapen et al. 2011). The lower matching of the graft and the higher dose of TNC are necessary. Now it is considered that UCBT should be selected with a higher than 4/6 HLA match and dose of 3–5 $\times$ $10^7$ TNC/kg depending on the results of HLA compatibility. Moreover, the incompatibility of HLA C increases the rates of transplant related mortality (TRM)(Eapen et al. 2011); thus, ideally UCB should match 7/8 or 8/8.

In order to multiply stem cells, several clinical procedures are currently in clinical use. Studies by Horwitz et al. have shown that ex vivo application of nicotinamide can extremely improve outcomes of UCBT (Horwitz et al. 2014). The recent finding by Cohen et al. has revealed that UM171 can be successfully used for stem cell enhancement (Cohen et al. 2020). There are more multiplication boosters that are still under further investigation, such as copper chelator, mesenchymal stromal cells, delta-like ligand 1 (DL-1), Stem Regenin-1 (SR-1), notch-mediated expansion, aryl hydrocarbon inhibition, stem cell renewal agonist, CD3/CD28 co-stimulation, and automated continuous perfusion (Horwitz et al. 2014; Cohen et al. 2020; Wagner et al. 2016; Delaney et al. 2010; de Lima et al. 2012, 2008; Stiff et al. 2018; Jaroscak et al. 2003; Hexner et al. 2016; Anand et al. 2017; Mehta et al. 2017).

Another strategy is to enhance homing of the cells in vivo by manipulating the SDF-1-CXCR4 axis by inhibiting dipeptidyl peptidase 4 (DPP-4), incubating with prostaglandin E2 (PGE2), or enforcing fucosylation of UCB cells (Farag et al. 2013; Cutler et al. 2013; Popat et al. 2015). These agents ensure that more HSC could reach the BM niche.

HSC fucosylation in vitro is one of the perspective methods used to enhance homing of the cells. During inflammation, P-selectin and E-selectin act on leukocyte migration and adhesion to the desired target. After HSCT, P-selectin and E-selectin ensure that intravenously administered HSC migrates to the bone marrow. In many tissues, P-selectin and E-selectin are expressed selectively on endothelial cells, but they are continuously expressed on bone marrow endothelial cells. During transplantation, HSC is injected into a vein and affixes to the bone marrow for multiplying (homing). Homing depends

on the interaction of P- and E-selectin on endothelial cells with their ligands on HSCs. These selectins are membrane-bound C-type lectins, and their relevant ligands should be α1,3-fucosylated to form terminal glycan determinants. Thus, fucosylation of HSC may improve liaison between bone marrow endothelial cells and HSCs(Popat et al. 2015).

In addition, intra-bone transplantation of UCB has demonstrated lower rates of aGvHD, earlier platelet recovery, and lower relapse rate (Brunstein et al. 2009; Frassoni et al. 2010); thus, it is also a promising technique to improve outcomes of UCBT.

## 5.1 UCB Transplantation (UCBT) for Children

Keeping in mind the small amount of stem cells available in UCB, this transplantation procedure currently focuses predominantly on children as they need fewer stem cells owing to their lower body weight. Acute leukemia (AL) is the most frequent malignancy in the pediatric population; thus, refractory or relapsed AL is the most common indication for aHSCT. Several studies attempted to compare the outcomes of UCB vs. BM/PBSC transplantation for children. The results are similar and state that overall survival doesn't differ between UCBT and BMT, but UCBT has a higher risk of delayed engraftment and immunologic reconstitution, with lower risk of GvHD (Stiff et al. 2018; Rocha et al. 2001; Barker et al. 2001; Dalle et al. 2004). Interestingly, another analysis by Eapen et al. found that matched UCBT has a better leukemia-free survival (LFS) than BMT in children transplanted due to leukemia (Eapen et al. 2007). If the UCBT has one or two HLA mismatches, the LFS is the same in UCBT and BMT groups. Moreover, UCBT could be a better alternative for adult and children's leukemia with positive pretransplant MRD than MUD or mismatched unrelated donor (MMUD) (Milano et al. 2016). UCBT is associated with better CD4+ reconstitution which ensures lower rates of relapse in children with AML (Admiraal et al. 2016a). In vitro studies justify this finding by revealing better GVL effect of UCBT (Horwitz et al. 2014). The recent study by Keating et al. suggests that UCBT could be especially advantageous in treating childhood AML (Keating et al. 2019).

Although unrelated UCB transplants are an important alternative for MUD, it is rarely discussed about the use of UCB and relevance in related cord blood transplantation. As summarized above, the gold standard is aHSCT from siblings due to the genetic and immunological similarities of cells and lower possibility of GvHD. Unrelated UCB cells can also overcome immunologic disparity as they are immunologically naive. UCB transplantation is less reactive because it consists of antigen-inexperienced lymphocytes that hardly react to allo-stimulation. Moreover, UCB transplant has more Tregs that inhibit immunological reactions. These factors may be important for lower possibility of GvHD (Beksac 2016; Kim and Broxmeyer 2011; Kanda et al. 2012). But when we face a related UCB transplantation, both abovementioned features are combined. The comparative analysis of related UCB and MSD transplant showed that the 3-year survival rates were the same between two groups; however, neutrophil engraftment occurred later in the UCB group, but the incidence of aGvHD III–IV degree was higher in sibling group (Stavropoulos-Giokas et al. 2012; Rocha et al. 2000; Cohen and Nagler 2004). The summary of Eurocord has revealed similar results. Children treated with related UCB transplantation due to their hematologic malignancies (mostly acute leukemia) had an engraftment rate of 90% at day 60 and a low percent of aGvHD and cGvHD (12% and 10%, respectively) 2 years after aHSCT (Herr et al. 2010). However, the relapse incidence was high: 47% by 5 years after aHSCT; but it was related to the administration of GvHD prophylactic regimens (e.g., Mtx). Nevertheless, this fact suggests that related UCB may not have such strong GVL effect as unrelated UCB transplantation.

Regarding pediatric aHSCT, it is considered that even unrelated UCB is an important alternative to MUD depending on the urgency of transplantation, HLA matching, and collected cell dose (Delaney and Ballen 2010; Gluckman and

Rocha 2009). Current practice suggests that matched 6/6 or > 6/8 HLA UCB units with adequate TNC count should be considered in the pediatric population, especially for those pathologies where graft versus malignancy effect is not necessary or for high risk with positive pretransplant MRD leukemia (Mayani et al. 2020; Gabelli et al. 2020).

### 5.2 Alternative Effects of UCBC

UCB is abundant in Tregs cells (CD4 + CD25 + Foxp3+), proliferating in the presence of IL-2. They are the main immunomodulatory cells that ensure maternal-fetal immune tolerance (Leber et al. 2010; Tsuda et al. 2019). Tregs can be successfully isolated and expanded up to 100-fold adding IL-2 (McKenna et al. 2017). These cells could be extremely important in the prevention and treatment of GvHD. As described above, aGvHD is the main cause of posttransplant morbidity and mortality as it is an inflammatory condition that damages tissues and organs. Tregs could inhibit GvHD while preserving the ability of GVL effect (Edinger et al. 2003).

Virus-specific T cells (VST) derived from UCB are an emerging treatment option for viral reactivation after transplantation. Usually patients are faced with cytomegalovirus (CMV), Epstein-Barr virus (EBV), and adenovirus (ADV) viremia, which prompts active aggressive antiviral treatment. Cases where this conventional treatment fails or causes too many toxic reactions may be treated with antivirus-specific T cells (VST) (Gupta and Wagner 2020). Recently, a simplified novel technique has been introduced – a single culture of polyclone VST (Dave et al. 2017).

However, UCBT lacks the possibility after transplantation to use donor lymphocytes infusion (DLI) in order to induce GVL or to prevent graft rejection in mixed-chimerism nonmalignant disorders. But there are some reports of in vitro expanded lymphocytes obtained from small amounts of UCB before transplantation (Berglund et al. 2017).

A very promising field is UCB derived NK chimeric antigen receptor (CAR) therapy. UCB NK cells express checkpoint inhibitory receptors that play the main role in antitumor effect and prevent disease relapse after HSCT. NK cells are characterized by CD16+/CD56+ and are responsible for nonself-antigens without antigen specificity. They are the first cells that reconstitute after HSCT, exerting a positive effect on engraftment, but have less cytotoxic potential (Merindol et al. 2011; Beziat et al. 2009; Verneris and Miller 2009; Wang et al. 2007). The main problem to taking advantage of these effects is the low number of NK cells in UCB; thus, enhancement techniques provide a perspective for anticancer immunotherapy (McKenna et al. 2017; Zhang et al. 2013; Heinze et al. 2019; Nham et al. 2018). The recent conclusions of a phase I/II trial, conducted by Liu et al., have revealed that UCB derived NK-CAR cells for patients with CD19 + malignancies do not cause any cytokine release syndrome, neurotoxicity, or GvHD (Use of CAR-transduced natural killer cells in CD19-positive lymphoid tumors).

## 6 Therapeutic Properties of Umbilical Cord Mesenchymal Cells

UCB has been valuable mainly for hematopoietic and progenitor stem cells, but the posttransplant outcome may depend on other types of stem cells, e.g., mesenchymal cells, which are also abundant in fetal tissues (cord blood and cord tissue) (Umbilical cord-derived mesenchymal stem cells for hematopoietic stem cell transplantation). We will hence continue with further discussion of the properties and therapeutic use of these cells.

MCSs are multipotent stem cells with self-renewal and high proliferative capacity (Umbilical cord-derived mesenchymal stem cells for hematopoietic stem cell transplantation; Marino et al. 2019). They are distinguished by specific surface markers (negative for hematopoietic or endothelial cells and positive for mesenchymal and cell adhesion molecules) and have mainly a

trilineage differentiation potency: to osteoblasts, chondroblasts, or adipocytes (Dominici et al. 2006).

MSC may be classified as adult-type (e.g., BM, adipose tissue) or perinatal/fetal-type (fetus or extraembryonic – placenta, umbilical cord blood, Wharton's jelly, amniotic membrane) (Marino et al. 2019; Zuk et al. 2002; Abdulrazzak et al. 2010). However, BM remains the main source of MSC. The first experience using MSC to treat aGvHD was reported in 2004 in Sweden (Kelly and Rasko 2021; Le Blanc et al. 2004). Since then, numerous clinical trials have been conducted to determine the benefits of MSCs (Kelly and Rasko 2021). The majority of them includes BM as a MSC source, and some of them analyze cord blood or adipose tissue (Kelly and Rasko 2021).

There are several important differences in MSCs depending on the stem cell source. MSC from fetal tissues are known to have greater expansion and proliferation capacity (Selmani et al. 2008; Galleu et al. 2017; Spees et al. 2016), can be obtained and captured more efficiently (Secco et al. 2008), and express lower HLA class I, and there is absence of HLA DR (Zhang et al. 2009; Deuse et al. 2011) than in adult-type MCS. Thus, fetal-derived MSCs are less immunogenic and may escape recognition of the alloreactive immune system (Umbilical cord-derived mesenchymal stem cells for hematopoietic stem cell transplantation) and have greater immunosuppressive effect (Wu et al. 2011). In addition, fetal-type (e.g., umbilical cord, especially Wharton's jelly) MSCs can be obtained more easily than MSCs from BM. After the delivery, UCMSCs are taken without any interventional procedure or risk for mother and baby (Secco et al. 2008; Lu et al. 2006). Moreover, MSCs from BM are prone to aging and losing their primary features of proliferation and differentiation (Stenderup et al. 2003).

MCSs are significant in improving outcomes after HSCT in several ways. Firstly, MSCs provide a microenvironment for HSC. They ensure expansion of HSC by expressing essential cytokines, adhesion molecules, and extracellular matrix proteins (Lazennec and Jorgensen 2008; Deans and Moseley 2000; Ito et al. 2006). In 2000, the first study that analyzed the results of co-transplantation of HSC and MSCs was performed and revealed better engraftment rates than of HSC transplantation alone (Koç et al. 2000). Interestingly, several studies have shown that faster engraftment was achieved using UCMSC rather than BMMSC (Chao et al. 2011).

Another important feature of MSCs is the possible prevention and treatment of GvHD. GvHD is caused by the graft's T cells attacking the recipient's body. Various inflammation cytokines and activated immune cells dominate in the disease pathophysiology and cause damage of the tissues (e.g., skin, liver, gastrointestinal tract) (Umbilical cord-derived mesenchymal stem cells for hematopoietic stem cell transplantation). The first choice of GvHD treatment remains corticosteroids (Kelly and Rasko 2021); however, around 50% of cases are resistant to these regimens. The 2-year overall survival rates of steroid resistant GvHD is only up to 20% (Westin et al. 2011). There are several options for second line treatment; a wide range of them are under investigation, but no agent has been identified as optimal (Kelly and Rasko 2021). MSCs may decrease the clinical manifestation of GvHD or even prevent it due to immunomodulatory and immunosuppressive features. Secreted cytokines (mainly indoleamine 2,3-dioxygenase – IDO) decrease the proliferation of alloreactive T cells (Galipeau and Sensébé 2018; Harrell et al. 2019; Terness et al. 2002). Moreover, Tregs expansion is induced and then the reactions to alloantigens are suppressed (Wagner et al. 2005). During apoptosis, MSCs release apoptotic extracellular vesicles that also have an immunomodulatory effect (Selmani et al. 2008). In addition, apoptosis induces IDO production in recipient's phagocytes (Galleu et al. 2017). Due to MSCs' paracrine effects, they may limit tissue damage that is caused by GvHD (Spees et al. 2016). These cells also may modulate innate and cellular immune pathways (B cells, NK, monocytes,

dendritic cells) (Umbilical cord-derived mesenchymal stem cells for hematopoietic stem cell transplantation; Weiss and Dahlke 2019).

Lastly, there are ongoing clinical trials on MSCs' antiviral properties after HSCT. The common complication after aHSCT is the reactivation of viruses (e.g., EBV, CMV, ADV, herpes virus (HSV)). In the early phase of viral infection, MSCs produce a strong immune response by releasing anti-inflammatory cytokines, attracting leukocytes to the site of injury and regulating the functions of all immune cells that are involved in the antiviral response (DCs, NK cells, macrophages, B lymphocytes, CD4+ helper, CTL cells) (Harrell et al. 2019; Gazdic et al. 2015). After viral elimination, MSCs produce immunoregulatory cytokines that suppress the excessive activation of the immune system and help to avoid a cytokine storm (Volarevic et al. 2017). Trophic factors support the repair of tissues (Sleem and Saleh 2020; Thanunchai et al. 2015). Nonetheless, there are still safety issues that need to be determined. As MSCs express receptors that are used by viruses (HIV, EBV, herpes) for their interaction with target cells, it is thought that MSCs may transmit viruses for patients undergoing HSCT (Cheng et al. 2013; Rollín et al. 2007; Soland et al. 2014). Though the study of Sundin et al. did not find any DNA derived from CMV, EBV, HSV-1 and HSV-2, or varicella from seropositive healthy donors, intracellular antigens can be found after infection of CMV and HSV type 1 in vitro (Sundin et al. 2006). Thus, this reciprocal interaction of MSCs and viruses needs to be investigated further, in order to make a conclusion about the benefits of MSC therapy for viral infections after HSCT.

To date, there is an agreement that MSCs are safe and well tolerated and usually do not cause any serious side effects except for transient fever (Galderisi et al. 2021; Sharma et al. 2014). It is known that MSCs of the donor may graft in the recipient's BM, but the stroma remains host in origin (Umbilical cord-derived mesenchymal stem cells for hematopoietic stem cell transplantation; Chao et al. 2011; Ball et al. 2007; Villaron et al. 2004; Poloni et al. 2006; Awaya et al. 2002). It means that after some time MSCs are eliminated from the recipient's body. Thus, more than one MSC infusion may be needed to treat GvHD; however, the therapy outcome is not related to the amount of infusions but correlates with the earlier time of MSC therapy (Marino et al. 2019).

As MSCs are a relatively new therapy, more prospective studies are needed to define therapeutic indications, cell sources, optimal dose, and frequency.

## 7 UCB Cells in Regenerative Medicine

Although UCB is mainly used for transplantation to treat blood disorders, the range of treatable diseases is expanding. Scientists apply umbilical cord blood or tissue in regenerative cell therapy or immune modulation. Purified cell populations could also facilitate possible gene therapy when there is a need to select HSC with the transgene inserted into the desired chromosomal location (Expansion of human cord blood hematopoietic stem cells for transplantation 2010).

UC MSC can replace damaged cells due to their ability to regenerate and differentiate. In addition, paracrine factors can inhibit programmed host cell death, modify immunological functions, and promote endogenous SC proliferation and differentiation (a primary mechanism). The reciprocal interaction between UC MSC and host cells plays a crucial role in current therapeutic approaches (Li et al. 2015).

**Host Cell Replacement** There are two ways to change cells: transdifferentiation and cell fusion. Transdifferentiation refers to the ability of MSC to differentiate into different cell types. Cell fusion refers to the fusion between a MSC and a host cell that allows nuclear reprogramming: to express specific genes for MSC and apoptosis-protected host cells (Li et al. 2015).

**Paracrine Factors** UC MSC can secrete a variety of growth factors, cytokines, and chemokines (including hepatocyte growth factor, stromal

factor 1 and monocyte chemotactic protein 1, vascular endothelial growth factor, insulin-like growth factor 1, interleukin-8, brain neurotransmitter glial cell-derived neurotrophic factor). These factors can help to prevent apoptosis of adjacent cells, promote angiogenesis, modulate inflammation, and activate internal SC, which helps to create a favorable environment for internal restorative processes (Li et al. 2015).

**Cell-Cell Contacts** UC MSCs are in a microenvironment where they can interact with host cells through rigid junctions, gap junctions, and desmosomes to affect host cell proliferation, migration, and differentiation. There is a need for a detailed understanding of the possible reciprocal interaction between umbilical cord MSC and host cells. As a result, it may help us with an alternative concept to decide the fate of transplanted cells and help us elucidate cell therapy strategies (Li et al. 2015).

Today, there are over 180 clinical trials registered on *clinicaltrials.gov*, investigating the effects of UCMSC in regenerative medicine. The application of MSC mainly focuses on the treatment of neurologic, autoimmune, cardiovascular, pulmonary, hepatic, and orthopedic diseases. The most prominent effects are seen in childhood neurology. Autologous cord blood infusion for children with cerebral palsy obviously improves motor and mental function (Sun et al. 2017). Effects of allogeneic cord blood and tissue cells on neurologic functions are also explored, and later phases of clinical trials will reveal more information (Sun et al. 2021).

## 8 Conclusions and Recommendations for Future Research

UCB SC is an attractive source for HSCT due to its rapid availability, noninvasive collection, lower risk of GVHD, and less stringent HLA matching. UCB is an alternative for MUD for children depending on the urgency of transplantation, HLA matching, and collected cell dose. UCBT should be selected with a higher than 4/6 HLA match and dose of 3–5 $\times$ $10^7$ TNC/kg depending on the results of HLA compatibility. Regarding the adult population, UCB should be selected when there is no MSD or MUD available and transplant is needed urgently. dUCB is as effective as sUCB in the adult population However, new techniques of HSC expansion and better homing are emerging that may overcome the issue of small amounts of HSC in one unit.

Special emphasis should be placed on cases with positive pretransplant MRD – UCBC – which have been shown to have better GVL effect and lower risk of relapse than MUD or MMUD in both pediatric and adult populations. The main disadvantages of the UCBC are delays in engraftment and immune recovery, thus higher risk of infections. But now it is also known that recovery is associated with conditioning regimens, especially ATG. Thus, special attention should be paid to ATG exposure during the peritransplantation period in order to avoid GvHD and to restore the immune system more quickly. In the near future, UCBC may be adopted as an important tool to alleviate complications after HSCT, such as Tregs, to suppress GVHD or virus-specific T cells to combat viral infections. A highly promising field is UCB derived NK-CAR therapy, as it shows no cytokine reactions.

In addition, umbilical cords contain not only hematopoietic stem cells but also mesenchymal stem cells. These cells may also facilitate the procedure of HSCT by increasing engraftment rates of HSC. They also may prevent and treat GvHD as well as viral reactivation posttransplantation. There are many ongoing clinical trials that are investigating the properties and therapeutic potential of UC hematopoietic and mesenchymal cells. MSC therapeutic properties for regenerative purposes are expanding rapidly, and it is expected that they will soon be approved by the regulatory agencies and brought into clinical practice.

In summary, the ethical acceptability and clinical features of umbilical cord blood and tissue mean that they may offer a wide range of novel

approaches for the prevention and treatment of disease.

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