Chapter

Umbilical Cord Blood and Cord Tissue Bank as a Source for Allogeneic Use

Tokiko Nagamura-Inoue and Fumitaka Nagamura

Abstract

Recently, umbilical cord blood (CB) has received attention as the allogeneic optimum source for immunotherapies. More recently, the umbilical cord (UC) has been rapidly utilized as an abundant source of mesenchymal stromal cells (MSCs), which migrate toward the inflammatory and damaged tissue to subside the inflammation and support tissue repair. Both CB and UC can be provided “off-the-shelf” cell products for immunotherapies and regenerative medicine. As biomedical wastes, CB and UC can be obtained noninvasively without any risks to the donor. CB cells and UC-derived MSCs (UC-MSCs) also have higher proliferation potentials than other cells obtained from adult tissues. In addition, UC-MSCs are less immunogenic and have significant immunosuppressive ability. Several clinical trials with CB or UC-MSCs have been conducted based on these advantages. The establishment of a stable supply system of CB and UC-MSCs is critical now for their utilization in regenerative and immune cell therapies. We have thus established the cord blood/cord bank, “IMSUT CORD,” as a new type of biobank, to supply both frozen CB and UC tissues and derived cells for research and clinical uses. In this chapter, we will introduce the overall flow from collection to shipment and discuss several issues that need to be resolved in unrelated allogeneic stable supply system.

Keywords: cord blood, umbilical cord, mesenchymal stromal cells, immune cell therapy, regenerative medicine, biobank, explant method, large-scale expansion

1. Introduction

Umbilical cord blood (CB) has been utilized as a source of hematopoietic stem cells for three decades. It could potentially also serve as the optimum source of immune cells, such as mononuclear cells (MNC), regulatory T cells, NK cells, and mesenchymal stromal cells (MSCs) with or without genetic modifications, for immunotherapy and neurogenic regeneration in some cases. In addition, UB could be prepared as readily available products [1].

It is well-known that human mesenchymal stromal cells (MSCs) can be harvested from various tissues that include the bone marrow (BM) [2], cord blood (CB) [3], adipose tissue [4], placenta [5], and umbilical cord (UC) [6]. Recently, clinical trials using MSCs for various diseases have been conducted, and some of them were approved. The BM is considered the traditional source of MSCs, and the characteristics and applications of BM-derived MSCs (BM-MSCs) have
been studied in detail. However, the harvesting of these cells is associated with an invasive procedure, and it may cause hemorrhage, infection, and chronic pain. In addition, BM-MSCs exhibit accelerated senescence as the donors’ age [7].

On the other hand, both CB and UC are routinely discarded as medical waste. The harvesting of CB and UC-derived MSCs (UC-MSCs) is therefore noninvasive and painless. The CB drawn from the UC and placenta is well-known as the source of hematopoietic stem cells for CB transplantations. However, in this article, we focus on the CB as the source for immune cells and regenerative medicine, such as regulatory T cells (Treg), NK cells, MSCs, and so on. The UC is the conduit between the developing embryo and placenta and consists of two umbilical arteries and one umbilical vein buried in the Wharton’s jelly. UC-MSCs have the abilities of multipotency and self-renewal properties comparable or superior to MSCs derived from other tissues in some papers. For this reason, several private CB banks have begun to collect CB and UC. We have thus established the cord blood/cord bank, “IMSUT CORD”, as a new type of biobank, to supply both frozen UC tissues and master cells for research and clinical uses.

In this chapter, we will introduce the overall flow from collection to shipment as taking the example of IMSUT CORD and discuss several issues that need to be resolved in unrelated allogeneic off-the-shelf stable supply system at present.

2. Cord blood and umbilical cord collection

There are many public and private CB banks in the world, in which procedures are nearly standardized intended for hematopoietic stem cell transplantation (HSCT) as shown in Section 5. The procedures include informed consent acquisition from the mother, collection of CB, processing, storage, to shipment, which have been already established. In the case of UC bank for unrelated allogeneic uses, the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) which issued ICH Q5A as the regulation of materials for biological products requires the second blood test from the baby’s mother, to deny viral infection in window period at delivery. Figure 1 shows the overall process of banking in the mother’s side. We deal with both CB and UC. In

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**Figure 1.**
Overall flow from informed consent acquisition to shipment.
the CB and UC collection hospitals, the purpose, overall process, private information policy, the right to withdrawal, 6-month health check, and second blood test for the mother to confirm the negative study of infection are explained to the mother, and she gives written consent as the guardian of the baby. In addition to obtaining informed consent, questionnaires about medical history, genetic history of the baby donor’s family, and history for the mother’s communicable disease risk behavior are conducted to survey their health. The CB and UC are then collected, and the mother’s blood is tested for infections. These documentations and tests in CB bank can be referred to UC banking as well, although additional safety tests for UC banking shall be required strictly. UC-MSCs from one donor can be delivered and administered to many patients. Especially when the CB and UC passed the safety and some quality tests at clinical grade, the mother is asked to receive the blood test to make sure that all infection tests are negative in 6 months after delivery. These second tests are demanded by the Pharmaceuticals and Medical Devised Agency (PMDA) like the FDA and EMA, because it should be proven that the donor’s mother and baby were not in the window period of viral infections at delivery. On the other hand, bacterial contamination is also taken care because the baby and placenta with UC come out from nonsterile vagina. We collect UC in the case of a scheduled cesarean section to reduce the possibility of contamination due to the exposure to the vaginal bacterial/fungal flora.

CB and UC are then transported from the collection hospitals to the CB/UC bank under controlled and validated temperature. CB is transported at validated temperature range (2–25°C) to protect cell viability, and the UC is cooled at 2–10°C in our facility.

3. Cord blood processing

Among the processing methods to obtain nucleated cells from CB for hematopoietic stem cell transplantation, the hydroxyethyl starch (HES) centrifugation method (HES method) is the most efficient and common. The HES method originated from the New York Blood Center CB bank [8]. Recently, automated CB processing SEPAX® (GE Healthcare Life Sciences) [9] and AutoXpress Platform® (Cesca Therapeutics, Inc.) [10] have been developed. For CB cryopreservation, DMSO and Dextran 40 together with CB-plasma are used worldwide [8].

On the other hand, no processing method of fresh or frozen CB not for hematopoietic stem cell transplantation has been settled as standard. The use of mononuclear cells (MNCs) obtained by the Ficoll-Paque centrifugation method (Ficoll method) or cell sorting with antibody-conjugated magnetic beads might be a new candidate for further processing and culture method. CB processed by HES method resulted in whole nucleated cells including neutrophils, monocytes, lymphocytes, and nucleated red blood cells with some amount of red blood cells (RBC). The recovery rate of hematopoietic stem cells and mononuclear cells processed by HES method is superior to those by Ficoll method. That is why HES method is introduced by CB banks in the world [8]. However, neutrophils in the nucleated cells and residual RBC may cause the aggregation resulting in the difficulty of further processing, when the frozen and thawed cells are diluted with large volume of isotonic solutions such as medium. Only frozen-thawed CB nucleated cells can be diluted with dextran and albumin/saline solution [8]. On the other hand, frozen-thawed MNCs processed by Ficoll method does not require such a special solution and can be diluted with medium and PBS, although the recovery rate of MNCs from the fresh CB by Ficoll method is less than that by HES methods.
MSCs derived from fresh CB are difficult to expand. Only one company, Medipost Co., Ltd., in Korea, has succeeded in expanding CB-derived MSCs. Their product, Cartistem®, has been approved by the Ministry of Food and Drug Safety in Korea for the treatment of osteoarthritis [11].

4. Umbilical cord processing

There are diverse procedures and culture methods for the isolation of MSCs from the various compartments of UC, such as Wharton's jelly, veins, arteries, UC lining membrane, subamnion, and perivascular regions [12]. The isolation methods of MSCs from the Wharton's jelly, vein, and arteries of UC are reported previously, although the marked differences were not found as far as the 10% fetal bovine serum (FBS) and α minimum essential medium (MEM) [13]. There are several papers to obtain MSCs from whole UC versus Wharton's jelly [14] or different parts of the same UC [15], but we suggest that to process from whole UC seems sufficient and simple for further processing [15]. Despite the wide range of isolation and culture procedures, the different groups seem to agree on the cryopreservation of UC tissue [16] and explant method [17] followed by the harvest of migrating cells from tissue. However, large-scale culture methods remain to be determined. Figure 2 shows the example of scheme of CB and UC collection and process and shipping to clinical use.

4.1 Cryopreservation of UC tissue

It is known that the UC tissue can be frozen in a cryoprotectant. This possibility of cryopreservation is the advantages of UC tissue for both clinical and research uses. The reasons of the advantages are:

1. UC tissue processing can be started after the donor’s health and infection statuses are confirmed well. This leads to initial cost-effectiveness because unnecessary works using inappropriate materials are eliminated. In addition, we can thaw a small amount of the UC to survey, before culturing MSCs in a large scale.

2. Storage of the tissues of origin allows us to keep traceability and to check the quality as the biological resources at a later date.

3. When new reagents or techniques were developed in the future, we can isolate novel cells from the cryopreserved UC tissues.

4. If the donor, the baby, has diseases that can be treated with autologous cells, including iPSC cells or gene-modified cells, or autologous UC-MSCs, the cryopreserved UC tissues would be the appropriate source.

Several animal serum-free cryoprotectants containing 5–10% dimethyl sulfoxide (DMSO) are available. Whether the use of serum originating from animals, such as fetal bovine serum (FBS), is required, is critical, because it adds the risk of the transmission of zoonotic infections, immunological reactions, and additional regulatory issues [18]. There are several reports of cryopreservation of the UC tissue, using serum-free and xenogeneic animal-free (xeno-free) cryoprotectants. Ennis et al. introduced CryoStor CS10® (BioLife Solutions Inc., WA) for isolating human UC perivascular cells (HUPVCs). However, they did not show the comparative test results to those of fresh UC [19]. Roy et al. reported the cryopreservation of the
UC tissue in 10% DMSO and 0.2 M sucrose solution, but the cumulative cell yield derived from the frozen-thawed UC-MSCs in their solution was inferior to that of fresh UC-MSCs [20]. We previously reported the cryopreservation of UC tissue, with no impact on viability, using a serum- and animal origin-free cryoprotectant, STEM-CELLBANKER® [16]. We demonstrated that cells derived from UC cryopreserved in this manner retained the phenotypic characteristics of MSCs, including the immunosuppressive activity in allogeneic mixed lymphocyte reactions, as well as differentiation potential. As shown in Figure 2, with the cryopreservation of UC tissue, UC processing might be altered.

4.2 Improved explant method

There are two major approaches after frozen-thawed UC tissue: explant and enzymatic digestion methods. Frozen-thawed UC tissue is manually minced into small fragments approximately 1–2 mm³ in size. Mincing is preferred to using a surgical scalpel or the use of an autologous mixer. These fragments are aligned and seeded regularly on a tissue culture-treated dish. After the tissue fragments attach to the bottom of the dish, culture media is added, slowly and gently in order not to detach the fragments [21–24]. Media is then refreshed every 3–7 days for 2–4 weeks until the fibroblast-like adherent cells reach 80–90% confluence. Subsequently, adherent cells and tissue fragments are rinsed once with PBS, detached using trypsin, and washed with media. The culture is then filtered to remove tissue fragments. The disadvantage in the explant method is that tissue fragments often float in media, resulting in the poor recovery of cells. To protect the exfoliation of tissue fragments from the bottom of the culture dish, we introduced stainless steel mesh (Cellamigo®; Tsubakimoto Chain Co.) shown in Figure 2, No. 8. In this manner, we can plate source tissue more quickly and harvest more MSCs. In addition, the incubation time required to reach 80–90% confluence is reduced [17].

In the enzymatic digestion method, UC is minced into small pieces and immersed in the media containing enzymes such as collagenase, or a combination of collagenase and hyaluronidase with or without trypsin [21, 24–26]. The cells dissociated by the enzymes are then cultured until they reach full confluence.
However, the digestion method is costly and time-consuming and may result in decreased cell viability due to lytic activity and varying sensitivity of the cells to collagenase. In addition, the initial harvested cells include more of the other types of cells compared with that harvested using the explant method.

4.3 Large-scale expansion and harvesting the cells

It is critical to consider how much we can expand the UC-MSCs to allow allogeneic “off-the-shelf” industrial availability, because the proliferation of adherent cells needs a large surface area. The conventional method uses multilayered flasks, and the cells are cultured in incubators installed in cleanrooms. These multilayered flasks can consistently support the expansion of UC-MSCs, and the state of cell confluence can be examined under the microscope. However, this method requires the considerable involvement of operators because the processes of seeding, refreshment, passage, and harvest require individual and manual works. Several companies have introduced the spinner bioreactor with a microcarrier made of plastic, dextran, denatured collagen-coated beads, and other components. The bioreactor system may reduce the number of operators required and may allow to reduce the clean levels of facility since it is a closed system. On the contrary, several critical problems of the bioreactor system exist. The cost of equipment is high and it is difficult to evaluate cell proliferation environment such as pH, lactate, and so on. When some microcarriers are torn off by spinner, or undigested microcarriers are residual in the final products, we have no ideas to remove the residual microcarriers completely. Recently, a plastic bag bioreactor system with a microcarrier in gentle locking was reported [27]. The most critical problem is that the cells produced by the flask-based culture method may be different from those by bioreactors. Harvesting cells on a large scale is still not easy. Recently, filter-based cell concentration and washing systems were introduced (https://www.kaneka.co.jp/en/business/healthcare/med_006.html, KANEKA, Japan). Automatic cell packaging may be also required in large-scale expansion.

The academic culture level such as IMSUT CORD is at small to middle scale. Only the company may have the ability to expand the cells at extra-large scale and maintain to control and supply the cell product for clinic constantly.

4.4 Long-term cryopreservation

Master and product cells of UC-MSCs for clinical use are usually required for long-term cryopreservation, together with records on the donor infant and the mother. There are several cryoprotectants for long-term cryopreservation. The most popular cryoprotectant consists of 5–10% dimethyl sulfoxide with human albumin. Recently, serum-free cryoprotectants, described in Section 4.1, have been commercialized and are thought to be more ideal compared to those containing human-derived serum. In addition to cryoprotectants, it is important to build an adequate record preservation system. Those who manage the long-term cryopreservation should preserve the records that include the documentation relating to the collection including donors, processing, results of quality tests, and instruments directly related to the products. The kinds of records and the length to be preserved are in accordance with the bank policies and standards and the corresponding domestic laws and regulations. It is necessary to discuss how long we should or we can cryopreserve CB and UC tissue, UC master cells, and product cells, in the technical and ethical aspects. In the technical aspect, the cell-preserving vessel to accommodate the cell suspension
for long-term freezing should be durable in a liquid nitrogen. In the ethical aspect, we do not expect whether the babies can recapture their ownership of CB and UC even though their mother waived the ownership of them, when they grow up to be adult.

### 4.5 Quality and safety assurance of UC-MSCs

The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy proposed the minimal criteria for defining human MSCs [28, 29]. First, MSCs must be plastic-adherent when maintained in standard culture conditions. Second, MSC must express CD105, CD73, and CD90 but not CD45, CD34, CD14 or CD11b, CD79α or CD19, and HLA-DR surface molecules. Third, MSCs must differentiate into adipocytes, chondroblasts, and osteoblasts in vitro [30–32]. Immunosuppressive effects have now become the most popular property of MSCs for potential clinical use [12]. Defect in HLA-class II expression with negative CD80 and CD86 in UC-MSCs even in the presence of inflammatory cytokines such as IFN-γ can theoretically rescue them from immune recognition by CD4+ T cells [33]. MSCs can also inhibit proliferation and cytokine secretion by immune cells, as well as alter subtypes of macrophage from M1 to M2 in vitro [34–37]. This immunomodulation is linked mainly to soluble factors such as indoleamine 2,3-dioxygenase (IDO), PGE2, and HLA-G5 [38], hepatocyte growth factor, and transforming growth factor-β1 released from MSCs [39]. Further quality tests are dependent on clinical applications and characteristics of MSCs.

The safety tests required differ according to the risks of clinical applications. For example, the tests of CB banking for hematopoietic stem cell transplantation are different from those of UC-MSCs. Donor-recipient relation of the former is one-to-one, and the risk is limited to one patient. On the other hand, that relationship of the latter, as UC-MSCs master cells and product cells, is one-to-many, so hundreds of patients may suffer health injuries by one donor. Thus, the vials of UC-MSCs are tested thoroughly at a designated time not only known viruses but also unknown viruses. Those tests should follow the local, national or international applicable laws and regulations. More precise safety tests for CB and UC shall be described elsewhere for the respective products for clinical application.

### 5. Standards and guidance for CB and UC from collection to release

There are international standards/guidance for CB collection, banking, and release of hematopoietic stem cell transplantation, such as the Foundation for the Accreditation of Cellular Therapy (FACT)/NETCORD [40], American association of Blood Banks (AABB), US Food and Drug Administration (FDA) shown in Table 1, and local standards or regulations under the applicable laws in respective countries. A CB/UC bank, facility, or individual should implement if the standard of practice in the community or applicable law establish additional requirements. International standards/guidance for biobanking process for UC collection, processing, culture, and release has not been settled, but collection and banking protocols can follow the CB banking standards and good tissue practice. Each CB/UC bank, facility, and individual should analyze its practices and procedures to determine whether additional standards apply. Compliance with the standards is not an exclusive means of complying with the standard of care in the industry or community or with local, national, or international laws or regulations [40]. Allogeneic public CB banks requested US FDA accreditation with FACT/NetCord or AABB in the USA, while the CB banks in Europe (EU) required FACT/NetCord
| Items                                                                 | Accreditation organization | Standards or guidance titles                                                                 |
|----------------------------------------------------------------------|-----------------------------|------------------------------------------------------------------------------------------------|
| Cord blood (CB) processing for hematopoietic stem cell transplantation| FACT/NETCORD                | International Standards for Cord Blood Collection, Banking, and Release for Administration     |
|                                                                      | FACT/JACIE                  | International Standards for Hematopoietic Cellular Therapy Product Collection, Processing, and |
|                                                                      |                             | Administration                                                                                |
|                                                                      |                             | http://www.factwebsite.org/cbstandards/                                                       |
| FDA in the USA                                                       |                             | Guidance for Industry: Minimally Manipulated, Unrelated Allogeneic Placental/Umbilical Cord   |
|                                                                      |                             | Blood Intended for Hematopoietic Reconstitution for Specified Indications                     |
|                                                                      |                             | Guidance for Industry and FDA Staff: Investigational New Drug Applications for Minimally     |
|                                                                      |                             | Manipulated, Unrelated Allogeneic Placental/Umbilical Cord Blood Intended for Hematopoietic   |
|                                                                      |                             | and Immunologic Reconstitution in Patients with Disorders Affecting the Hematopoietic System  |
|                                                                      |                             | http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/   |
|                                                                      |                             | default.htm                                                                                    |
| AABB                                                                |                             | Standards for Cellular Therapy Services                                                          |
|                                                                      |                             | http://www.aabb.org/sa/Pages/Standards-Portal.aspx                                              |
| Umbilical cord-derived cells including mesenchymal stromal cells    | FACT                        | Common standards for Cellular Therapies                                                          |
| (UC-MSCs) / somatic cell or other derivative cells CB not intended    |                             | http://www.factwebsite.org/cbstandards/                                                        |
| for hematopoietic stem cell transplantation                          |                             | FDA in the USA                                                                                 |
|                                                                      |                             | Good Tissue Practice 21CFR 1271.210 Current Good Tissue Practice (CGTP) and Additional         |
|                                                                      |                             | Requirements for Manufacturers of Human Cells, Tissues, and Cellular and Tissue-Based Products  |
|                                                                      |                             | Guidance for Industry: Preclinical Assessment of Investigational Cellular and Gene Therapy     |
|                                                                      |                             | Products                                                                                       |
|                                                                      |                             | Guidance for FDA Reviewers and Sponsors Content and Review of Chemistry, Manufacturing, and     |
|                                                                      |                             | Control (CMC) Information for Human Somatic Cell Therapy Investigational New Drug Applications  |
|                                                                      |                             | (INDs)                                                                                         |
|                                                                      |                             | http://www.fda.gov/cber/guidelines.htm                                                         |
| AABB                                                                |                             | Standards for Cellular Therapy Services                                                          |
|                                                                      |                             | http://www.aabb.org/sa/Pages/Standards-Portal.aspx                                              |
| EMA in EU                                                            |                             | Tissues and Cells Directives: Guideline on human cell-based medicinal products (EMEA/CHMP/   |
|                                                                      |                             | 410896/2006) for ATMP                                                                          |
| PMDA (Japan)                                                        |                             | Good Gene, Cellular, and Tissue-based Products Manufacturing Practice (GCTP)                     |
| Quality management system                                            | ISO                         | ISO9001                                                                                        |
|                                                                      |                             | ISO/TC276 (Draft)                                                                              |
|                                                                      |                             | https://www.iso.org/standard/62085.html                                                         |

Foundation for the Accreditation of Cellular Therapy, FACT; US Food and Drug Administration, FDA; American Association of Blood Banks, AABB; advanced therapy medicinal products, Pharmaceutical and medical devices agency (PMDA). This table does not include the law defined in each country. These standards, guidance, guidelines, and practices are not intended to apply all cell therapies using CB and UC. The CB/UC bank carefully chooses and implements them for your intended products under the applicable law.

Table 1.
Standards or guidance related to cord blood and umbilical cord-derived cells.
with additional requirements like FACT/JACIE standards, when it is requested by the respective national regulation affairs. There are many private or private-public combined CB banks in the world, which tend to follow the AABB standards and have the inspection and accreditation (http://www.aabb.org/sa/facilities/celltherapy/Documents/AABB-Accredited-Cord-Blood-Facilities.pdf).

6. Special issues of CB and UC bank system for allogeneic use

The number of clinical trials using CB and UC-MSCs in the fields of immune cell therapies and regenerative medicine has been increasing. On the other hand, CB as a source of hematopoietic stem cell transplantation is less used recently, because the cell number is limited, the engraftment of HSC is delayed, and HLA haplo-identical HSCT is induced and controlled. These clinical trials are aimed uses that include severe acute graft-versus-host disease (GVHD) treatment, rapid engraftment of HSCT, and the prevention of severe acute GVHD. Clinical trials using CB- and UC-MSCs are summarized in Tables 2 and 3, respectively. We started a sponsor-investigator clinical trial using UC-derived MSCs for patients with treatment-resistant severe acute GVHD supported by the research fund of the Japan Agency for Medical Research and Development (AMED). Consistent supply is the critical key to conduct clinical trials and for marketing. For the stable supply of frozen CB and UC, or UC-derived MSCs, we have established a CB and UC bank, named IMSUT CORD, in our institute. This bank also provides CB and UC-MSCs for immunotherapy and regenerative medicine products to hospitals and pharmaceutical companies shown in Figure 2. The bank also provides frozen CB, frozen UC, master cells, and the cells after master cells as an intermediate products requiring further processing or more culture in the companies.

The following are also the major points for managing CB and UC banking.

First, to build an adequate quality management system to serve the resource of cell therapy products, we have introduced the concept of the ISO 9001 and obtained its certification, and as a result, we introduced the concept of PDCA cycle. Second, involvements of various kinds of specialists must be considered. There are many procedures, such as collection, obtaining informed consent, application to ethics review committee, and document management. Third, health check and infection test of the donor’s mother are required to ensure that no infection is detected after window period of infections. In this process, both traceability and personal information protection must be satisfied. Fourth, we respect the right of decision to donate, rejection, or withdrawal. Donor’s mother should be explained the policy of the bank that the consented withdrawal time is set at the initiation of processing for clinical use. Although the CB and UC belong to the baby, we obtain informed consent from the donor’s mother as guardianship and ownership are asked to be transferred to the bank. Fifth, there is also the issue of how long the UC tissue and UC-MSCs can be cryopreserved. For example, in the Japanese public CB bank for HSCT, the CB is cryopreserved for 10 years as a clinical grade of HSC source. After this period, they are used for basic research or preclinical studies or discarded if they are not used for research. A cryopreservation period of 10 years for UC and UC-MSCs may be the first threshold to be checked. In addition, we disclose the information in website for the mothers who have not been explained about the new researches or new clinical trials at the first IC acquisition. Lastly, because unlike CB, the UC is a tissue considered as a part of the perinatal appendage, we must follow the tissue transport and medical disposal/waste regulations under the applicable laws or local rules and ethical standards.
| Authors         | Cell type                      | Disease                                                                 | Patients number | Age (range) year | Route and procedure of administration | Cell number/kg or body | Results                                                                 | Adverse events                                      |
|-----------------|-------------------------------|------------------------------------------------------------------------|-----------------|-----------------|----------------------------------------|------------------------|----------------------------------------------------------------------|----------------------------------------------------|
| Brunstein et al. [79] | CB-NC-derived Treg             | Grade II–IV acute GVHD                                                  | Treg: 11        | 61 (46–68)      | IV                                     | 3–100 × 10⁶ Treg/kg     | aGVHD: Treg group 9%, control 45% cGVHD: Treg 0%, control 14%        | No dose-limiting infusion adverse events            |
|                  | (CB from The New York Blood Center) |                                                                 |                 |                 |                                        |                        |                                                                      |                                                    |
| Control: 22      |                               |                                                                        |                 |                 |                                        |                        |                                                                      |                                                    |
|                  |                               |                                                                        |                 |                 |                                        |                        |                                                                      |                                                    |
| Kellner et al. [80] | Fucosylated or non-fucosylated UCB-Tregs | HSCT                                                             | 5               |                 | IV (–1 day of HSCT)                    | 1 × 10⁶/kg             | —                                                                    | No infusion reactions                               |
| Zhu et al. [81]  | CB-MNC                        | Chronic complete spinal cord injury                                    | 8 in Hong Kong  | 42.6 ± 2.7      | IT (dorsal entry zone)                 | 1.6–3.2 × 10⁶           | Walk 10 m, 15/20 pts. (p = 0.001), no necessity of assistance for bladder management, 12/20 (p = 0.001) and bowel management (p = 0.002) | 1 neuropathic pain; 1 subarachnoid hematoma and pneumocephalus due to cerebrospinal loss; 1 arachnoid hemorrhage IHK group, 68 AEs including postoperative wound swelling; 9 pain Overall 5 severe AE in 28 patients |
| Shah et al. [82] | CB-MNC-derived NK cells (CB from MD Anderson Cord Blood Bank) | Multiple myeloma undergoing autologous PBSCT                         | 12              | 48–70           |                                        | 5 × 10⁶, 1 × 10⁶, 5 × 10⁷ and 1 × 10⁸ CB-NK cells/kg | 10 achieved VGPR (8 near CR) as the best response | No infusional toxicities and no GVHD                   |
| Lv et al. [83]   | CB-MSC + UC-MSCs              | Autism                                                                 | 14 CB-MNC       |                 | IV                                     | Proximately 2 × 10⁷/kg CB-MNCs, 1 × 10⁷/kg of UC-MSCs 4 times in 5–7 day | Improvement of CARS, ABC scores, and CGI evaluation at 24 weeks in CB-MNCs with UC-MSCs | No treatment related and no severe adverse effects |

| Cell number/kg or body | Results | Adverse events                                      |
|------------------------|---------|----------------------------------------------------|
| 3–100 × 10⁶ Treg/kg    | aGVHD: Treg group 9%, control 45% cGVHD: Treg 0%, control 14% | No dose-limiting infusion adverse events |
| 1 × 10⁶/kg             | —       | No infusion reactions                               |
| 1.6–3.2 × 10⁶          | Walk 10 m, 15/20 pts. (p = 0.001), no necessity of assistance for bladder management, 12/20 (p = 0.001) and bowel management (p = 0.002) | 1 neuropathic pain; 1 subarachnoid hematoma and pneumocephalus due to cerebrospinal loss; 1 arachnoid hemorrhage IHK group, 68 AEs including postoperative wound swelling; 9 pain Overall 5 severe AE in 28 patients |
| 5 × 10⁶, 1 × 10⁶, 5 × 10⁷ and 1 × 10⁸ CB-NK cells/kg | 10 achieved VGPR (8 near CR) as the best response | No infusional toxicities and no GVHD |
| Proximately 2 × 10⁷/kg CB-MNCs, 1 × 10⁷/kg of UC-MSCs 4 times in 5–7 day | Improvement of CARS, ABC scores, and CGI evaluation at 24 weeks in CB-MNCs with UC-MSCs | No treatment related and no severe adverse effects |
### Table 2.
Clinical trials using allogeneic cord blood.

| Authors          | Cell type                  | Disease                          | Patients number | Age (range) year | Route and procedure of administration | Cell number/kg or body | Results                                                                 | Adverse events            |
|------------------|----------------------------|----------------------------------|-----------------|-----------------|----------------------------------------|------------------------|--------------------------------------------------------------------------|---------------------------|
| Dolstra et al. [84] | CB-CD34-derived NK cells (CB from Cord Blood Bank Nijmegen) | AML in old patients             | 10              | 68–76           | IV                                     | 3 and 30 × 10⁷/kg       | NK cell maturation in vivo, MRD become negative in 2/4 with MRD before IV | No GVHD, no toxicity     |
| Park et al. [85]  | CB-derived MSCs           | Rheumatoid arthritis             | 9               | 57.4 ± 10.0     | IV                                     | 2.5 × 10⁷, 5 × 10⁷, or 1 × 10⁸ | DAS2/ESR decreased, inflammatory cytokine levels are reduced | No DLT, no major toxicity |
| Laskowitz et al. [86] | CB-NC (CB from Carolinas Cord Blood Bank or MD Anderson Cord Blood Bank) | Cerebral stroke                  | 10              | 65.5 (45-79)    | IV on 3–9 days poststroke              | Cell dose 1.54 (0.84-3.34) × 10⁷ kg, CD34⁺ 2.03 (0.10-6.80) × 10⁵/kg | All improved by at least one grade in Modified Rankin Score | AE tolerated no serious AE |
| Huang et al. [87] | CB-MSCs                   | Cerebral palsy (age: 3–12)       | 27              | 29.07 ± 2.03 (n = 14) | IV                                     | 2.5 × 10⁷                | Improved atopic dermatitis scores, pruritus score, serum IgE and eosinophil number | No serious AE               |
| Kim et al. [88]   | CB-MSCs                   | Moderate-to-severe atopic dermatitis | 34 (7 in phase I, 27 in phase IIa) | 28.08 ± 1.07 (n = 11) | SC                                     | 5.0 × 10⁷                |                                                                                  | No serious AE               |

**AE**, adverse event; **AML**, acute myeloid leukemia; **CB**, cord blood; **UC**, umbilical cord; **MSCs**, mesenchymal stromal cells; **MNCs**, mononuclear cells; **NK cells**, natural killer cells; **Treg**, regulatory T cells; **GVHD**, graft-versus-host disease; **PBSCT**, peripheral blood stem cell transplantation; **IV**, intravenous injection; **SC**, subcutaneous injection.
| Authors       | Disease                                         | Patients number | Age (range) year | Route and procedure of administration | Cell number/kg or body | Frequency interval | Results                  | Adverse events |
|---------------|-------------------------------------------------|-----------------|------------------|----------------------------------------|------------------------|-------------------|--------------------------|----------------|
| Wu et al.     | Severe steroid-resistant aGVHD                  | 2               | Pt 1: 4          | IV                                     | Pt 1: 3.3, 7.2, 8.0 × 10^6/kg | 3                 | Improved                 | No             |
|               |                                                 |                 | Pt 2: 6          | IV                                     | Pt 2: 4.1 × 10^6/kg     | 1                 |                          |                |
| Si et al.     | Severe aplastic anemia                          | 37              | 5                | IV (7–10 days after HSCT)              | 1 × 10^6/kg            | 1                 | aGVHD II–IV; 17 of 37 (45.9%) | No             |
| Wu et al.     | Refractory/relapsed hematologic malignancy      | 50              | 26 (9–58)        | IV (4 h before haploidentical HSCT)    | 5 × 10^6/kg            | 1                 | aGVHD II–IV; 12 of 50 (24.0%); cGVHD, 17 of 45 (37.7%) (3 extended) | No             |
| Wu et al.     | Severe AA                                        | 21              | 18 (4–31)        | IV (4 h before HSCT)                  | 5 × 10^6/kg            | 1                 | aGVHD II–IV; 12 of 21 (57.1%); 3 of 9 extended cGVHD | No             |
| Fu et al.     | Refractory severe AA                            | 5               | 15.2 (9–22)      | IV (2 days after PBSCT)               | 1 × 10^6/kg            | 1                 | No severe aGVHD or cGVHD | No             |
| Gao et al.    | Prophylaxis of chronic GVHD after HLA-haploidentical stem cell transplantation | 62              | Age < 8, 15 pts.; 18–40, 39; >40, 8 | IV                                     | 3 × 10^5 cells         | Until cGVHD occurred, leukemia relapsed, or 4 cycles | No             |
| Zhu et al.    | High-risk acute leukemia                        | 25              | 11.2 (4–17)      | IV (before haploidentical HSCT)        | Median 1.14 × 10^6/kg (1.03–1.39 × 10^6/kg) | 4 (over 7 days intervals) | aGVHDI, 8 of 25 (32.0%); cytomegalovirus viremia, 23 of 25 (92.0%) | No             |
| Authors          | Disease                                              | Patients number | Age (range) year | Route and procedure of administration | Cell number/kg or body | Frequency interval | Results                  | Adverse events |
|------------------|------------------------------------------------------|-----------------|------------------|----------------------------------------|------------------------|-------------------|--------------------------|----------------|
| Pan et al. [48]  | Extensive bone marrow necrosis of a chronic myeloid leukemia patient | 1               | 10               | iBM                                    | iBM: 2 × 10^7/kg       | 1                 | BM recovered             | No             |
|                  |                                                      |                 |                  | IV                                     | IV: 2 pp. × 10^6/kg    |                   |                          |                |

**Neurogenic injuries**

| Authors      | Disease                                | Patients number | Age (range) year | Route and procedure of administration | Cell number/kg or body | Frequency interval | Results                               | Adverse events |
|--------------|----------------------------------------|-----------------|------------------|----------------------------------------|------------------------|-------------------|----------------------------------------|----------------|
| Wang et al.  [49] | Traumatic brain injury                 | 20              | 27.5 (5–48)      | Intrathecal (IT)                       | 1 × 10^7               | 4 (5–7 days intervals) | Motor functional recovery after 6 months | No             |
| Jin et al.   [50]  | Hereditary spinocerebellar ataxia      | 16              | 39.9 (21–56)     | IV + intrathecal                       | IV; 4 × 10^7           | 4 (over 7 days interval) | Motor functional recovery after 6 months | No             |
| Wang et al.  [51]  | Cerebral palsy                         | 16 (8 twins)   | 6.29 (3–12)      | IT                                     | 1–1.5 × 10^7 cells     | 4 (3–5 days intervals) | Motor functional recovery after 1 and 6 months | No             |

**Diabetes mellitus**

| Authors      | Disease                                | Patients number | Age (range) year | Route and procedure of administration | Cell number/kg or body | Frequency interval | Results                                                                 | Adverse events |
|--------------|----------------------------------------|-----------------|------------------|----------------------------------------|------------------------|-------------------|-------------------------------------------------------------------------|----------------|
| Guan et al.  [52] | DM (type 2)                            | 6               | 40.5 (27–51)     | IV                                     | 1 × 10^6/kg            | 2 (2 weeks interval) | Insulin-independent for 25–43 Mo, 3 dose reduction of insulin, others | No             |
| Hu et al.    [53]  | DM (type 1)                            | 15              | 17.6             | IV                                     | 2.6 ± 1.2 × 10^7/kg    | 2 (4 weeks interval) | HbA1c and C-peptide improvement in MSCs group                            | No             |
| Cai et al.   [54]  | DM (type 1)                            | 21              | 18–29 (5–28) at onset | Supraselective pancreatic artery cannulation | 1.1 × 10^6/kg, with autologous BM-MNC | 1                 | Moderate improvement of metabolic measures                               | 1 transient abdominal pain |
| Authors               | Disease                                      | Patients number | Age (range) year | Route and procedure of administration | Cell number/kg or body | Frequency interval | Results                                                      | Adverse events                      |
|-----------------------|----------------------------------------------|-----------------|------------------|----------------------------------------|------------------------|-------------------|-------------------------------------------------------------|-------------------------------------|
| Kong et al. [55]      | DM (type 2)                                  | 18              |                  | IV                                     | $1 \times 10^6$/kg     | Day 0 and until Day 90 if effective | FBS reduced plasma C-peptide and regulatory T cells increased | 4/18: slight fever                  |
|                       |                                              |                 |                  |                                        |                        |                   |                                                             |                                     |
|                       |                                              |                 |                  |                                        |                        |                   |                                                             |                                     |
| Heart and angioplasty |                                              |                 |                  |                                        |                        |                   |                                                             |                                     |
| Cai et al. [56]       | Avascular necrosis of the femoral head       | 30              | 41.6 (19–63)     | Femoral head artery (co-transplant with autologous BM) | Autologous BM-BM-MNCs, $60.7 \pm 11.5 \times 10^6$/kg UC-MSCs, $1 \times 10^6$/kg | 1                 | Improved                                                   | No                                  |
|                       |                                              |                 |                  |                                        |                        |                   |                                                             |                                     |
|                       |                                              |                 |                  |                                        |                        |                   |                                                             |                                     |
| Can et al. [57]       | Myocardial ischemia                          | 39              | 30–80            | Intracoronary                          | $2 \times 10^7$/kg     | 1                 | Ongoing                                                     | No                                  |
|                       |                                              |                 |                  |                                        |                        |                   |                                                             |                                     |
|                       |                                              |                 |                  |                                        |                        |                   |                                                             |                                     |
| Zhao et al. [58]      | Severe systolic heart failure                | 30              | 52.9 (20–79)     | Intracoronary                          | Unknown                | 1                 | Cardiac remodeling and function improved with reduced mortality rate | No                                  |
|                       |                                              |                 |                  |                                        |                        |                   |                                                             |                                     |
|                       |                                              |                 |                  |                                        |                        |                   |                                                             |                                     |
| Li et al. [59]        | Coronary chronic total occlusion             | 15              | Unknown          | Intracoronary                          | $3 \times 10^7/4 \times 10^7/5 \times 10^7$/kg | 1                 | Infarcted size reduced with improved left ventricular EF     | No                                  |
|                       |                                              |                 |                  |                                        |                        |                   |                                                             |                                     |
|                       |                                              |                 |                  |                                        |                        |                   |                                                             |                                     |
| Mustalek et al. [60]  | Acute myocardial infarction                  | 10              | 55.6 (32–65)     | Intracoronary                          | $3 \times 10^7$/body   | 1                 | Feasible and procedural safe as off-the-shelf cellular therapy | Transient fever (38.9°C)             |
|                       |                                              |                 |                  |                                        |                        |                   |                                                             |                                     |
| Authors          | Disease                                      | Patients number | Age (range) year | Route and procedure of administration | Cell number/kg or body | Frequency interval | Results                                                                 | Adverse events |
|------------------|----------------------------------------------|-----------------|------------------|----------------------------------------|------------------------|-------------------|-------------------------------------------------------------------------|----------------|
| Bartolucci [61]  | Heart failure                                | 15              | 57.33 ± 10.05    | IV                                     | $1 \times 10^6$ cells/kg | 1                 | Significant improvements in LVEF, NYHA functional class, Minnesota Living with Heart Failure Questionnaire | No             |
| Liver            |                                               |                 |                  |                                        |                        |                   |                                                                         |                |
| Xue et al. [62]  | Decompensated liver cirrhosis                | 50              | Unknown          | Intrahepatic artery                    | $3 \times 10^7$/body   | 1                 | Increase of serum albumin                                               | No             |
| Wang et al. [63] | Primary biliary cirrhosis                    | 7               | 49 (33–58)       | IV                                     | $5 \times 10^7$/kg at 4 weeks interval | 3                 | ALP and γ-GTP                                                           | No             |
| Shi et al. [64]  | Prevention of acute liver allograft rejection | 14 (13, single dose, 1 multiple dose) | 57 ± 12          | IV                                     | $1 \times 10^6$ cells | Single (13 pts), 3 times every 4w (1 pt) | Decreases of ALT, AST, T-BIL, Histologic improvements, MSCs 6, control 0 | No             |
| Liang et al. [65]| Liver cirrhosis caused by autoimmune diseases | 23 (2 CB-MSC, 1 BM MSC) | 53.4 (35–70)    | IV                                     | $1 \times 10^6$ cells/kg | 1                 | Not statistically significant improvement                               | 2, fever; 3, mild fidgetiness, suffered from insomnia |                |
| Zhang et al. [66]| Ischemic-type biliary lesions following liver transplantation | 12              | 47.3 ± 10.1      | IV                                     | $1 \times 10^6$ cells/kg | 6 (1, 2, 4, 8, 12, 16 weeks) | Significantly decreased need for interventional therapies: 1-, 2-yr graft survival rates: MSCs group (100%, 83.3%), control group (72.9%, 68.6%) | No             |
| Authors       | Disease                              | Patients number | Age (range) year | Route and procedure of administration | Cell number/kg or body | Frequency interval | Results                                      | Adverse events                  |
|---------------|--------------------------------------|-----------------|------------------|----------------------------------------|------------------------|-------------------|----------------------------------------------|----------------------------------|
| Xu et al. [67]| Hepatitis B virus-related acute-on-chronic liver failure | 30:UC-MSC       | UC-MSC: 40.67 ± 9.89 | IV                                     | 10^6 cells/kg          | UC-MSC, once a week, 4 times | No significant improvement of short-term prognosis | Fever, UC-MSC 11 pts., PE + UC-MSC 6 pts |
|               |                                      | 20, UC-MSC/ plasma exchange | UC-MSC/ plasma exchange, 42.00 ± 6.55 | IV                                     | UC-MSC/ PE: first 2, UC-MSC: 2nd day after 1st, 3rd PE treatments | | |
| Zhang et al. [68] | Crohn's disease                      | 41              | 32.7 (20–41)     | IV                                     | 1 × 10^6 cells/kg      | Once a week, four times | Decreases of CDAI, HBI, corticosteroid dosage | Fever 4, upper respiratory tract infection, 7 |
| Hu et al. [69] | Ulcerative colitis                   | 34              | 42.9 ± 2.31      | IV then IA                             | 0.5 × 10^6 cells/kg   | 2, 7 days          | Decreases of median Mayo score, histology score, Improvement of IBDQ scores | No |
| Hashemi et al. [70] | Chronic skin ulcer                  | 5               | 30–60            | Covered by acellular amniotic membrane seeded with WJSCs | About 2 × 10^6 cells were seeded | Epithelial surface of acellular amniotic membrane | Significantly decreased wound healing time, wound size. Significantly declined wound size after 6, 9 days | Not stated |
| Authors            | Disease                                      | Patients number | Age (range) year | Route and procedure of administration | Cell number/kg or body | Frequency interval | Results                                      | Adverse events                      |
|--------------------|----------------------------------------------|-----------------|------------------|---------------------------------------|------------------------|-------------------|---------------------------------------------|-------------------------------------|
| **Kidney**         |                                              |                 |                  |                                       |                        |                   |                                             |                                     |
| Sun et al. [71]    | Prevention of delayed graft function and acute rejection in renal transplantation | 21              | 41.0 ± 11.5      | IV                                    | 2 × 10⁶ cells/kg (before transplantation), 5 × 10⁶ (during surgery) | ←                 | No significant improvement                  | No                                  |
| **Autoimmune diseases** |                                              |                 |                  |                                       |                        |                   |                                             |                                     |
| Deng et al. [72]   | Lupus nephritis                             | 12 MSC, 6 placebo | 29 ± 10          | IV                                    | 1 × 10⁹ cells         | 2 times 1 wk. interval | Not statistically significant improvement | 1: leucopenia, pneumonia, subcutaneous abscess, 1: severe pneumonia |
| Wang et al. [73]   | Active and refractory SLE                    | 40              | 17–54            | IV                                    | 1 × 10⁵ cells/kg on day 0 and 7 | 2                 | MCR (13 of 40, 32.5%), PCR (11 of 40, 27.5%) during 12 months, although several patients relapse after 6 months | No                                  |
| Wang et al. [74]   | RA                                           | 136             | 46.1             | IV                                    | 4 × 10⁷ cells, 2nd in 3 months later | 1 (n = 112)         | Decreases of serum TNF-α, IL-6, increase of regulatory T cells. Significant remission for 3–6 months | Mild fever (<38.5°C) without treatment at injection, 6 patients |
| Authors          | Disease                  | Patients number | Age (range) year | Route and procedure of administration | Cell number/kg or body | Frequency interval | Results                                                                                                            | Adverse events |
|------------------|--------------------------|-----------------|------------------|----------------------------------------|------------------------|-------------------|------------------------------------------------------------------------------------------------------------------|----------------|
| Riordan et al. [75] | Multiple sclerosis       | 20              | 41.2 (24–55)     | IV                                     | $2 \times 10^7$ UC-MSC | 7 (1–4 days)      | Significant improvements of various symptoms. Inactive lesions by MRI in 15/18 patients. (83.3%) after 1 year | Headache, fatigue |
| He et al. [76]    | Severe sepsis            | 15 (3 cohorts)  | 56 (25–72)       | IV                                     | $1 \times 10^6$ cells/kg | 1                 | System clinical outcomes are not changed                                                                   | No             |
| Cao et al. [77]   | Recurrent intrauterine adhesions | 27          | 35.1 ± 3.8 (27–42) | Loaded onto a collagen scaffold       | $1 \times 10^7$        | 1                 | Pregnant, 10 of 26 patients                                                                                     | No             |

aGVHD, acute graft-versus-host disease; cGVHD, chronic GVHD; HSCT, hematopoietic stem cell transplantation; AA, aplastic anemia; BM, bone marrow; IT, intrathecal injection; AE, adverse event; AML, acute myeloid leukemia; CB, cord blood; UC, umbilical cord; BM, bone marrow; PE, plasma exchange; RA, rheumatoid arthritis; MSCs, mesenchymal stromal cells; DM, diabetes mellitus; FBS, fast blood sugar; EF, ejection fraction; IV, intravenous injection; SC, subcutaneous injection; DM, diabetes mellitus.

Table 3. Clinical trials using allogeneic umbilical cord-derived mesenchymal stromal cells.
7. Private CB and UC banking for autologous and family use

Recently, there are an increasing number of private CB banks, which have initiated to serve the cryopreservation of UC, i.e., private CB and UC bank. Using private autologous CB, clinical trials for cerebral palsy caused by hypoxic ischemic encephalopathy (HIE) reported their efficacy [78], although the collection of CB is difficult for the baby in such a severe situation of delivery, resulting in the limited application entry. Recently, we obtained the proof of concept that the UC-MSCs attenuated the neurogenic and functional damage caused by intraventricular hemorrhage (IVH) in newborn model mice. Duke University implemented the clinical trial using allogeneic UC tissue-derived cells for the patients with HIE. Allogeneic off-the-shelf UC-MSCs are a promising source; however, we do not know the adverse events such as HLA antibody induction caused by long-term repeated injections of allogeneic cells. Therefore, autologous use of CB and UC is still challenged to be discussed continuously.

8. Conclusion

Although several problems still remain to be dissolved, operation of adequate CB and UC bank should be considered as the provider of cell source for regenerative and immune cell therapy, because of their prominent characteristics and convenient and noninvasive collection.

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Author details

Tokiko Nagamura-Inoue* and Fumitaka Nagamura

1 Department of Cell Processing and Transfusion, IMSUT CORD, Research Hospital, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan

2 Division of Advanced Medicine Promotion, The Advanced Clinical Center, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan

*Address all correspondence to: tokikoni@ims.u-tokyo.ac.jp

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References

[1] Liu E, Tong Y, Dotti G, et al. Cord blood NK cells engineered to express IL-15 and a CD19-targeted CAR show long-term persistence and potent antitumor activity. Leukemia. 2018;32(2):520-531

[2] Gnecchi M, Melo LG. Bone marrow-derived mesenchymal stem cells: Isolation, expansion, characterization, viral transduction, and production of conditioned medium. Methods in Molecular Biology. 2009;482:281-294

[3] Bieback K, Kluter H. Mesenchymal stromal cells from umbilical cord blood. Current Stem Cell Research & Therapy. 2007;2(4):310-323

[4] Gruber HE, Deepe R, Hoelscher GL, et al. Human adipose-derived mesenchymal stem cells: Direction to a phenotype sharing similarities with the disc, gene expression profiling, and coculture with human annulus cells. Tissue Engineering. Part A. 2010;16(9):2843-2860

[5] In ’t Anker PS, Scherjon SA, Kleijburg-van der Keur C, et al. Isolation of mesenchymal stem cells of fetal or maternal origin from human placenta. Stem Cells. 2004;22(7):1338-1345

[6] Romanov YA, Svititskaya VA, Smirnov VN. Searching for alternative sources of postnatal human mesenchymal stem cells: Candidate MSC-like cells from umbilical cord. Stem Cells. 2003;21(1):105-110

[7] Stenderup K, Justesen J, Clausen C, Kassem M. Aging is associated with decreased maximal life span and accelerated senescence of bone marrow stromal cells. Bone. 2003;33(6):919-926

[8] Rubinstein P, Dobrila L, Rosenfield RE, et al. Processing and cryopreservation of placental/umbilical cord blood for unrelated bone marrow reconstitution. Proceedings of the National Academy of Sciences of the United States of America. 1995;92(22):10119-10122

[9] Lapierre V, Pellegrini N, Bardey I, et al. Cord blood volume reduction using an automated system (Sepax) vs. a semi-automated system (Optipress II) and a manual method (hydroxyethyl starch sedimentation) for routine cord blood banking: A comparative study. Cytotherapy. 2007;9(2):165-169

[10] Solves P, Mirabet V, Blanquer A, et al. A new automatic device for routine cord blood banking: Critical analysis of different volume reduction methodologies. Cytotherapy. 2009;11(8):1101-1107

[11] Park YB, Ha CW, Lee CH, Yoon YC, Park YG. Cartilage regeneration in osteoarthritic patients by a composite of allogeneic umbilical cord blood-derived mesenchymal stem cells and hyaluronate hydrogel: Results from a clinical trial for safety and proof-of-concept with 7 years of extended follow-up. Stem Cells Translational Medicine. 2017;6(2):613-621

[12] Nagamura-Inoue T, He H. Umbilical cord–derived mesenchymal stem cells: Their advantages and potential clinical utility. World Journal of Stem Cells. 2014;6(2):195-202

[13] Ishige I, Nagamura-Inoue T, Honda MJ, et al. Comparison of mesenchymal stem cells derived from arterial, venous, and Wharton's jelly explants of human umbilical cord. International Journal of Hematology. 2009;90(2):261-269

[14] Mennan C, Brown S, McCarthy H, et al. Mesenchymal stromal cells derived from whole human umbilical cord exhibit similar properties to those derived from Wharton's jelly and bone marrow. FEBS Open Bio. 2016;6(11):1054-1066
[15] Bharti D, Shivakumar SB, Park JK, et al. Comparative analysis of human Wharton's jelly mesenchymal stem cells derived from different parts of the same umbilical cord. Cell and Tissue Research. 2018;372(1):51-65

[16] Shimazu T, Mori Y, Takahashi A, Tsunoda H, Tojo A, Nagamura-Inoue T. Serum- and xeno-free cryopreservation of human umbilical cord tissue as mesenchymal stromal cell source. Cytotherapy. 2015;17(5):593-600

[17] Mori Y, Ohshima J, Shimazu T, et al. Improved explant method to isolate umbilical cord-derived mesenchymal stem cells and their immunosuppressive properties. Tissue Engineering. Part C, Methods. 2015;21(4):367-372

[18] Lauterboeck L, Saha D, Chatterjee A, Hofmann N, Glasmacher B. Xeno-free cryopreservation of bone marrow-derived multipotent stromal cells from Callithrix jacchus. Biopreservation and Biobanking. 2016;14(6):530-538

[19] Ennis J, Gotherstrom C, Le Blanc K, Davies JE. In vitro immunologic properties of human umbilical cord perivascular cells. Cytotherapy. 2008;10(2):174-181

[20] Roy S, Arora S, Kumari P, Ta M. A simple and serum-free protocol for cryopreservation of human umbilical cord as source of Wharton's jelly mesenchymal stem cells. Cryobiology. 2014;68(3):467-472

[21] He H, Nagamura-Inoue T, Tsunoda H, et al. Stage-specific embryonic antigen 4 in Wharton's jelly-derived mesenchymal stem cells is not a marker for proliferation and multipotency. Tissue Engineering. Part A. 2014;20(7-8):1314-1324

[22] Majore I, Moretti P, Stahl F, Hass R, Kasper C. Growth and differentiation properties of mesenchymal stromal cell populations derived from whole human umbilical cord. Stem Cell Reviews. 2011;7(1):17-31

[23] Marmotti A, Mattia S, Bruzzone M, et al. Minced umbilical cord fragments as a source of cells for orthopaedic tissue engineering: An in vitro study. Stem Cells International. 2012;2012:326813

[24] Kandoi S, Kumar LP, Patra B, et al. Evaluation of platelet lysate as a substitute for FBS in explant and enzymatic isolation methods of human umbilical cord MSCs. Scientific Reports. 2018;8(1):12439

[25] Kikuchi-Taura A, Taguchi A, Kanda T, et al. Human umbilical cord provides a significant source of unexpanded mesenchymal stromal cells. Cytotherapy. 2012;14(4):441-450

[26] Salehinejad P, Alitheen NB, Ali AM, et al. Comparison of different methods for the isolation of mesenchymal stem cells from human umbilical cord Wharton's jelly. In Vitro Cellular & Developmental Biology. Animal. 2012;48(2):75-83

[27] Das R, Roosloot R, van Pel M, et al. Preparing for cell culture scale-out: Establishing parity of bioreactor- and flask-expanded mesenchymal stromal cell cultures. Journal of Translational Medicine. 2019;17(1):241

[28] Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006;8(4):315-317

[29] Horwitz EM, Le Blanc K, Dominici M, et al. Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement. Cytotherapy. 2005;7(5):393-395

[30] Wang JF, Wang LJ, Wu YF, et al. Mesenchymal stem/progenitor cells in human umbilical cord blood as support for ex vivo expansion of CD34(+) hematopoietic stem cells and for chondrogenic differentiation. Haematologica. 2004;89(7):837-844
[31] Karahuseyinoglu S, Cinar O, Kilic E, et al. Biology of stem cells in human umbilical cord stroma: In situ and in vitro surveys. Stem Cells. 2007;25(2):319-331

[32] Huang P, Lin LM, Wu XY, et al. Differentiation of human umbilical cord Wharton’s jelly-derived mesenchymal stem cells into germ-like cells in vitro. Journal of Cellular Biochemistry. 2010;109(4):747-754

[33] Weiss ML, Anderson C, Medicetty S, et al. Immune properties of human umbilical cord Wharton’s jelly-derived cells. Stem Cells. 2008;26(11):2865-2874

[34] Chiossone L, Conte R, Spaggiari GM, et al. Mesenchymal stromal cells induce peculiar alternatively activated macrophages capable of dampening both innate and adaptive immune responses. Stem Cells. 2016;34(7):1909-1921

[35] Deng Y, Zhang Y, Ye L, et al. Umbilical cord-derived mesenchymal stem cells instruct monocytes towards an IL10-producing phenotype by secreting IL6 and HGF. Scientific Reports. 2016;6:37566

[36] Cutler AJ, Limbani V, Girdlestone J, Navarrete CV. Umbilical cord-derived mesenchymal stromal cells modulate monocyte function to suppress T cell proliferation. Journal of Immunology. 2010;185(11):6617-6623

[37] Selleri S, Bifsha P, Civini S, et al. Human mesenchymal stromal cell-secreted lactate induces M2-macrophage differentiation by metabolic reprogramming. Oncotarget. 2016;7(21):30193-30210

[38] Seshareddy K, Troyer D, Weiss ML. Method to isolate mesenchymal-like cells from Wharton’s jelly of umbilical cord. Methods in Cell Biology. 2008;86:101-119

[39] Zhou C, Yang B, Tian Y, et al. Immunomodulatory effect of human umbilical cord Wharton’s jelly-derived mesenchymal stem cells on lymphocytes. Cellular Immunology. 2011;272(1):33-38

[40] (FACT) FFTAOCT. International Standards For Cord Blood Collection, Banking, And Release For Administration. 7th ed 2020

[41] Wu KH, Chan CK, Tsai C, et al. Effective treatment of severe steroid-resistant acute graft-versus-host disease with umbilical cord-derived mesenchymal stem cells. Transplantation. 2011;91(12):1412-1416

[42] Si Y, Yang K, Qin M, et al. Efficacy and safety of human umbilical cord derived mesenchymal stem cell therapy in children with severe aplastic anemia following allogeneic hematopoietic stem cell transplantation: A retrospective case series of 37 patients. Pediatric Hematology and Oncology. 2014;31(1):39-49

[43] Wu Y, Wang Z, Cao Y, et al. Cotransplantation of haploidentical hematopoietic and umbilical cord mesenchymal stem cells with a myeloablative regimen for refractory/relapsed hematologic malignancy. Annals of Hematology. 2013;92(12):1675-1684

[44] Wu Y, Cao Y, Li X, et al. Cotransplantation of haploidentical hematopoietic and umbilical cord mesenchymal stem cells for severe aplastic anemia: Successful engraftment and mild GVHD. Stem Cell Research. 2014;12(1):132-138

[45] Fu Y, Wang Q, Zhou J, et al. Reduced intensity conditioning and co-transplantation of unrelated peripheral stem cells combined with umbilical cord mesenchymal stem/stroma cells for young patients with refractory severe aplastic anemia. International Journal of Hematology. 2013;98(6):658-663

[46] Gao L, Zhang Y, Hu B, et al. Phase II Multicenter, randomized, double-blind
controlled study of efficacy and safety of umbilical cord-derived mesenchymal stromal cells in the prophylaxis of chronic graft-versus-host disease after HLA-haploidentical stem-cell transplantation. Journal of Clinical Oncology. 2016;34(24):2843-2850

[47] Zhu L, Wang Z, Zheng X, et al. Haploidentical hematopoietic stem cell transplant with umbilical cord-derived multipotent mesenchymal cell infusion for the treatment of high-risk acute leukemia in children. Leukemia & Lymphoma. 2015;56(5):1346-1352

[48] Pan Y, Wang X, Wang C, et al. Extensive bone marrow necrosis resolved by allogeneic umbilical cord blood mesenchymal stem cell transplantation in a chronic myeloid leukemia patient. Bone Marrow Transplantation. 2015;50(9):1265-1268

[49] Wang S, Cheng H, Dai G, et al. Umbilical cord mesenchymal stem cell transplantation significantly improves neurological function in patients with sequelae of traumatic brain injury. Brain Research. 2013;1532:76-84

[50] Jin JL, Liu Z, Lu ZJ, et al. Safety and efficacy of umbilical cord mesenchymal stem cell therapy in hereditary spinocerebellar ataxia. Current Neurovascular Research. 2013;10(1):11-20

[51] Wang X, Hu H, Hua R, et al. Effect of umbilical cord mesenchymal stromal cells on motor functions of identical twins with cerebral palsy: Pilot study on the correlation of efficacy and hereditary factors. Cytotherapy. 2015;17(2):224-231

[52] Guan LX, Guan H, Li HB, et al. Therapeutic efficacy of umbilical cord-derived mesenchymal stem cells in patients with type 2 diabetes. Experimental and Therapeutic Medicine. 2015;9(5):1623-1630

[53] Hu J, Yu X, Wang Z, et al. Long term effects of the implantation of Wharton's jelly-derived mesenchymal stem cells from the umbilical cord for newly-onset type 1 diabetes mellitus. Endocrine Journal. 2013;60(3):347-357

[54] Cai J, Wu Z, Xu X, et al. Umbilical cord mesenchymal stromal cell with autologous bone marrow cell transplantation in established type 1 diabetes: A pilot randomized controlled open-label clinical study to assess safety and impact on insulin secretion. Diabetes Care. 2016;39(1):149-157

[55] Kong D, Zhuang X, Wang D, et al. Umbilical cord mesenchymal stem cell transfusion ameliorated hyperglycemia in patients with type 2 diabetes mellitus. Clinical Laboratory. 2014;60(12):1969-1976

[56] Cai J, Wu Z, Huang L, et al. Cotransplantation of bone marrow mononuclear cells and umbilical cord mesenchymal stem cells in avascular necrosis of the femoral head. Transplantation Proceedings. 2014;46(1):151-155

[57] Can A, Ulus AT, Cinar O, et al. Human umbilical cord mesenchymal stromal cell transplantation in myocardial ischemia (HUC-HEART Trial). A study protocol of a phase 1/2, controlled and randomized trial in combination with coronary artery bypass grafting. Stem Cell Reviews and Reports. 2015;11(5):752-60

[58] Zhao XF, Xu Y, Zhu ZY, Gao CY, Shi YN. Clinical observation of umbilical cord mesenchymal stem cell treatment of severe systolic heart failure. Genetics and Molecular Research. 2015;14(2):3010-3017

[59] Li X, Hu YD, Guo Y, et al. Safety and efficacy of intracoronary human umbilical cord-derived mesenchymal stem cell treatment for very old patients with coronary chronic total occlusion. Current Pharmaceutical Design. 2015;21(11):1426-1432

[60] Musialek P, Mazurek A, Jarocha D, et al. Myocardial regeneration strategy
using Wharton’s jelly mesenchymal stem cells as an off-the-shelf ‘unlimited’ therapeutic agent: Results from the acute myocardial infarction first-in-man study. Postępy w Kardiologii Interwencyjnej. 2015;11(2):100-107

[61] Bartolucci J, Verdugo FJ, Gonzalez PL, et al. Safety and efficacy of the intravenous infusion of umbilical cord mesenchymal stem cells in patients with heart failure: A phase 1/2 randomized controlled trial (RIMECARD trial [Randomized clinical trial of intravenous infusion umbilical cord mesenchymal stem cells on cardiopathy]). Circulation Research. 2017;121(10):1192-1204

[62] Xue HL, Zeng WZ, Wu XL, et al. Clinical therapeutic effects of human umbilical cord-derived mesenchymal stem cells transplantation in the treatment of end-stage liver disease. Transplantation Proceedings. 2015;47(2):412-418

[63] Wang L, Li J, Liu H, et al. A pilot study of umbilical cord-derived mesenchymal stem cell transfusion in patients with primary biliary cirrhosis. Journal of Gastroenterology and Hepatology. 2013;28(Suppl 1):85-92

[64] Shi M, Liu Z, Wang Y, et al. A pilot study of mesenchymal stem cell therapy for acute liver allograft rejection. Stem Cells Translational Medicine. 2017;6(12):2053-2061

[65] Liang J, Zhang H, Zhao C, et al. Effects of allogeneic mesenchymal stem cell transplantation in the treatment of liver cirrhosis caused by autoimmune diseases. International Journal of Rheumatic Diseases. 2017;20(9):1219-1226

[66] Zhang YC, Liu W, Fu BS, et al. Therapeutic potentials of umbilical cord-derived mesenchymal stromal cells for ischemic-type biliary lesions following liver transplantation. Cytotherapy. 2017;19(2):194-199

[67] Xu WX, He HL, Pan SW, et al. Combination treatments of plasma exchange and umbilical cord-derived mesenchymal stem cell transplantation for patients with Hepatitis B virus-related acute-on-chronic liver failure: A clinical trial in China. Stem Cells International. 2019;2019:4130757

[68] Zhang J, Lv S, Liu X, Song B, Shi L. Umbilical cord mesenchymal stem cell treatment for Crohn’s disease: A randomized controlled clinical trial. Gut Liver. 2018;12(1):73-78

[69] Hu J, Zhao G, Zhang L, et al. Safety and therapeutic effect of mesenchymal stem cell infusion on moderate to severe ulcerative colitis. Experimental and Therapeutic Medicine. 2016;12(5):2983-2989

[70] Hashemi SS, Mohammadi AA, Kabiri H, et al. The healing effect of Wharton’s jelly stem cells seeded on biological scaffold in chronic skin ulcers: A randomized clinical trial. Journal of Cosmetic Dermatology. 2019;18(6):1961-1967

[71] Sun Q, Huang Z, Han F, et al. Allogeneic mesenchymal stem cells as induction therapy are safe and feasible in renal allografts: Pilot results of a multicenter randomized controlled trial. Journal of Translational Medicine. 2018;16(1):52

[72] Sun L, Wang D, Liang J, et al. Umbilical cord mesenchymal stem cell transplantation in severe and refractory systemic lupus erythematosus. Arthritis and Rheumatism. 2010;62(8):2467-2475

[73] Wang D, Zhang H, Liang J, et al. Allogeneic mesenchymal stem cell transplantation in severe and refractory systemic lupus erythematosus: 4 years of experience. Cell Transplantation. 2013;22(12):2267-2277

[74] Wang L, Wang L, Cong X, et al. Human umbilical cord mesenchymal
stem cell therapy for patients with active rheumatoid arthritis: Safety and efficacy. Stem Cells and Development. 2013;22(24):3192-3202

[75] Riordan NH, Morales I, Fernandez G, et al. Clinical feasibility of umbilical cord tissue-derived mesenchymal stem cells in the treatment of multiple sclerosis. Journal of Translational Medicine. 2018;16(1):57

[76] He X, Ai S, Guo W, et al. Umbilical cord-derived mesenchymal stem (stromal) cells for treatment of severe sepsis: A phase 1 clinical trial. Translational Research. 2018;199:52-61

[77] Cao Y, Sun H, Zhu H, et al. Allogeneic cell therapy using umbilical cord MSCs on collagen scaffolds for patients with recurrent uterine adhesion: A phase I clinical trial. Stem Cell Research & Therapy. 2018;9(1):192

[78] Cotten CM, Murtha AP, Goldberg RN, et al. Feasibility of autologous cord blood cells for infants with hypoxic-ischemic encephalopathy. The Journal of Pediatrics. 2014;164(5):973-979. e971

[79] Brunstein CG, Miller JS, McKenna DH, et al. Umbilical cord blood-derived T regulatory cells to prevent GVHD: Kinetics, toxicity profile, and clinical effect. Blood. 2016;127(8):1044-1051

[80] Kellner JN, Delemarre EM, Yvon E, et al. Third party, umbilical cord blood derived regulatory T-cells for prevention of graft versus host disease in allogeic hematopoietic stem cell transplantation: Feasibility, safety and immune reconstitution. Oncotarget. 2018;9(86):35611-35622

[81] Zhu H, Poon W, Liu Y, et al. Phase I-II clinical trial assessing safety and efficacy of umbilical cord blood mononuclear cell transplant therapy of chronic complete spinal cord injury. Cell Transplantation. 2016;25(11):1925-1943

[82] Shah N, Li L, McCarty J, et al. Phase I study of cord blood-derived natural killer cells combined with autologous stem cell transplantation in multiple myeloma. British Journal of Haematology. 2017;177(3):457-466

[83] Lv YT, Zhang Y, Liu M, et al. Transplantation of human cord blood mononuclear cells and umbilical cord-derived mesenchymal stem cells in autism. Journal of Translational Medicine. 2013;11:196

[84] Dolstra H, Roeven MWH, Spanholtz J, et al. Successful transfer of umbilical cord blood CD34(+) hematopoietic stem and progenitor-derived NK cells in older acute myeloid Leukemia patients. Clinical Cancer Research. 2017;23(15):4107-4118

[85] Park EH, Lim HS, Lee S, et al. Intravenous infusion of umbilical cord blood-derived mesenchymal stem cells in rheumatoid arthritis: A phase Ia clinical trial. Stem Cells Translational Medicine. 2018;7(9):636-642

[86] Laskowitz DT, Bennett ER, Durham RJ, et al. Allogeneic umbilical cord blood infusion for adults with ischemic stroke: Clinical outcomes from a phase I safety study. Stem Cells Translational Medicine. 2018;7(7):521-529

[87] Huang L, Zhang C, Gu J, et al. A randomized, placebo-controlled trial of human umbilical cord blood mesenchymal stem cell infusion for children with cerebral palsy. Cell Transplantation. 2018;27(2):325-334

[88] Kim HS, Lee JH, Roh KH, Jun HJ, Kang KS, Kim TY. Clinical trial of human umbilical cord blood-derived stem cells for the treatment of moderate-to-severe atopic dermatitis: Phase I/IIa studies. Stem Cells. 2017;35(1):248-255