Chapter

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

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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.

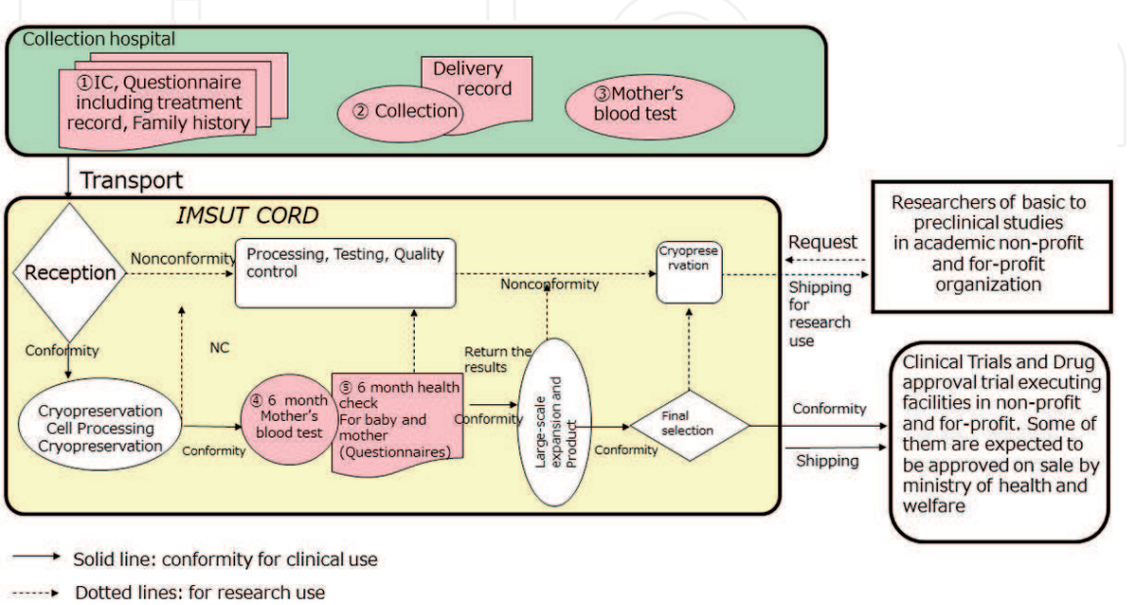

Figure 1.
Overall flow from informed consent acquisition to shipment.

In 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 iPS 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

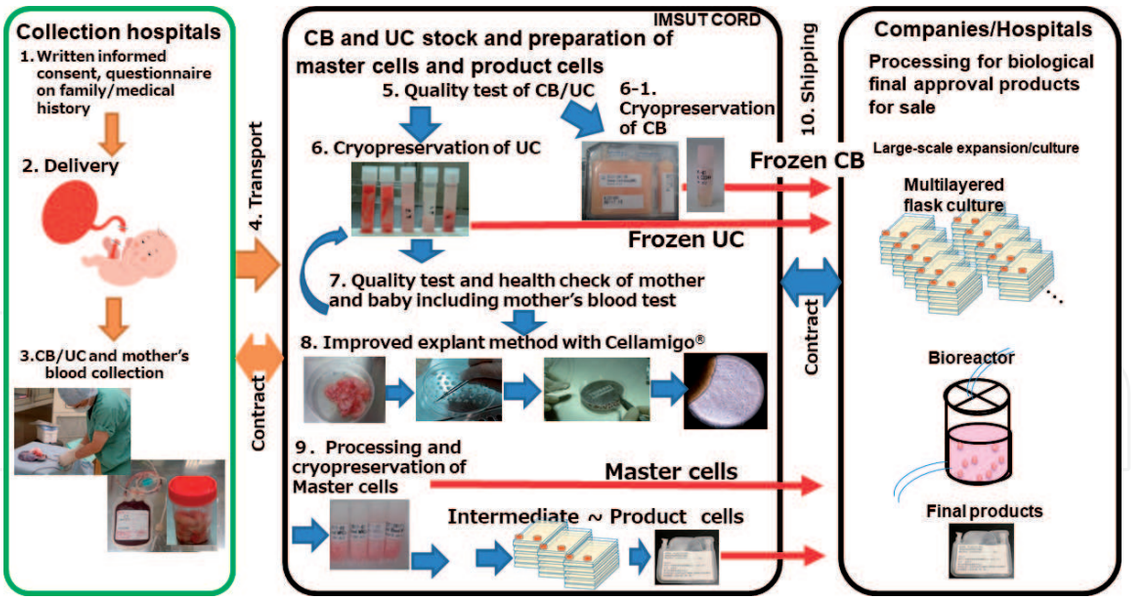

Figure 2.
IMSUT CORD scheme of CB and UC from collection to shipping for clinical use.

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 mm^3 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 multi-layered flasks, and the cells are cultured in incubators installed in cleanrooms. These multilayer 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 CD86in 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 of 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 FACT/JACIE	International Standards for Cord Blood Collection, Banking, and Release for Administration 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 (UC-MSCs)/somatic cell or other derivative cells CB not intended for hematopoietic stem cell transplantation	FACT	Common standards for Cellular Therapies http://www.factwebsite.org/cbstandards/
	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 (HCT/Ps) 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/cellthe-rapy/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 immunother-apy 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 cryopreserva-tion 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. [41]	CB-NC-derived Treg (CB from The New York Blood Center)	Grade II–IV acute GVHD	Treg: 11	61 (45–68)	IV	3–100 × 10^6 Treg/kg	aGVHD: Treg group 9%, control 45% cGVHD: Treg 0%, control 14%	No dose-limiting infusion adverse events
			Control: 22	60 (34–69)		—		
Kellner et al. [42]	Fucosylated or non-fucosylated UCB-Tregs	HSCT	5		IV (−1 day of HSCT)	1 × 10^6/kg		No infusion reactions
Zhu et al. [43]	CB-MNC	Chronic complete spinal cord injury	8 in Hong Kong	42.6 ± 2.7	IT (dorsal entry zone)	1.6–3.2 × 10^6	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 I HK group,
		Phase I–II	20 in Kunming	36.9 ± 2.4				68 AEs including postoperative wound swelling; 9 pain Overall 5 severe AE in 28 patients
Shah et al. [44]	CB-MNC-derived NK cells (CB from MD Anderson Cord Blood Bank)	Multiple myeloma undergoing autologous PBSCT	12	48–70		5 × 10^6, 1 × 10^7, 5 × 10^7 and 1 × 10^8 CB-NK cells/kg	10 achieved VGPR (8 near CR) as the best response	No infusional toxicities and no GVHD
Lv et al. [45]	CB-MSC + UC-MSCs	Autism	14 CB-MNC 9 CB-MNC and UC-MSCs 14 no cells therapy	CB-MNC: 7.08 (3.29–12.01) CB-MNC + UC-MSCs: 6.51 (3.98–9.83) Control: 5.02 (3.51–10.02)	IV	Proximately 2 × 10^6/kg CB-MNCs 1 × 10^6/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

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. [46]	CB-CD34-derived NK cells (CB from Cord Blood Bank Nijmegen)	AML in old patients	10	68–76	IV	3 and 30 × 10^6/kg	NK cell maturation in vivo, MRD become negative in 2/4 with MRD before IV	No GVHD, no toxicity
Park et al. [47]	CB-derived MSCs	Rheumatoid arthritis	9	57.4 ± 10.0	IV	2.5 × 10^7, 5 × 10^7, or 1 × 10^8	DAS2/-ESR decreased, inflammatory cytokine levels are reduced	No DLT, no major toxicity
Laskowitz et al. [48]	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) × 10^7/kg, $CD34^+$ 2.03 (0.10–6.80) × 10^5/kg	All improved by at least one grade in Modified Rankin Score	AE tolerated no serious AE
Huang et al. [49]	CB-MSCs	Cerebral palsy (age: 3–12)	27 (CB-MSCs)	CB-MSCs: 7 (3–12)	IV	27:4 CB-MSCs IV at 5 × 10^7 with basic rehabilitation treatment	Significant improved of GMFM-88 evaluation	No serious AE
			27 (control)	Control: 7 (3–12)				
Kim et al. [50]	CB-MSCs	Moderate-to-severe atopic dermatitis	34 (7 in phase I, 27 in phase IIa)	29.07 ± 2.03 (n = 14)	SC	2.5 × 10^7	Improved atopic dermatitis scores, pruritus score, serum IgE and eosinophil number	No serious AE
				28.08 ± 1.07 (n = 11)		5.0 × 10^7		

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.

Table 2.
Clinical trials using allogeneic cord blood.

Authors	Disease	Patients number	Age (range) year	Route and procedure of administration	Cell number/kg or body	Frequency interval	Results	Adverse events
Engraftment facilitation and graft-versus-host disease (GVHD) in hematopoietic stem cell transplantation								
Wu et al. [51]	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. [52]	Severe aplastic anemia	37	5 (0.75–11.58)	IV (7–10 days after HSCT)	1 × 10^6/kg	1	aGVHD II–IV; 17 of 37 (45.9%) cGVHD, 7 of 37 (18.9%)	No
Wu et al. [53]	Refractory/ relapsed hematologic malignancy	50	26 (9–58)	IV (4 h before haploidentical HSCT)	5 × 10^5/kg	1	aGVHD II–IV, 12 of 50 (24.0%) cGVHD, 17 of 45 (37.7%) (3 extended)	No
Wu et al. [54]	Severe AA	21	18 (4–31)	IV (4 h before HSCT)	5 × 10^5/kg	1	aGVHD II-IV;12 of 21 (57.1%) 3 of 9 extended cGVHD	No
Fu et al. [55]	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. [56]	Prophylaxis of chronic GVHD after HLA-haploidentical stem cell transplantation	62	Age < 8, 15 pts.; 18–40, 39; >40, 8	IV	3 × 10^7 cells	Until cGVHD occurred, leukemia relapsed, or 4 cycles	cGVHD at 2 yr.: MSCs group 27.4%, control 49.0% (P = .021). Severe lung cGVHD: MSCs group 0, control 7 (P = .047)	No
Zhu et al. [57]	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. [58]	Extensive bone marrow necrosis of a chronic myeloid leukemia patient	1	10	iBM IV	iBM: 2×10^7/kg IV: 2 pp. $\times 10^6$/kg	1	BM recovered	No
Neurogenic injuries								
Wang et al. [59]	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. [60]	Hereditary spinocerebellar ataxia	16	39.9 (21–56)	IV + intrathecal	IV; 4×10^7 IT; 2×10^7 cells	4 (over 7 days interval)	Motor functional recovery after 6 months	No
Wang et al. [61]	Cerebral palsy	16 (8 twins)	6.29 (3–12)	IT	$1–1.5 \times 10^7$ cells	4 (3–5 days intervals)	Motor functional recovery after 1 and 6 months	No
Diabetes mellitus								
Guan et al. [62]	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. [63]	DM (type 1)	15	17.6	IV	$2.6 \pm 1.2 \times 10^7$/kg	2 (4 weeks interval)	HbA1c and C-peptide improvement in MSCs group	No
Cai et al. [64]	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. [65]	DM (type 2)	18		IV	1×10^6/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. [66]	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, 1×10^6/kg	1	Improved	No
Can et al. [67]	Myocardial ischemia	39	30–80	Intracoronary	2×10^7/kg	1	Ongoing	No
Zhao et al. [68]	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. [69]	Coronary chronic total occlusion	15	Unknown	Intracoronary	$3 \times 10^6/4 \times 10^6/5 \times 10^6$/kg	1	Infarcted size reduced with improved left ventricular EF	No
Musialek et al. [70]	Acute myocardial infarction	10	55.6 (32–65)	Intracoronary	3×10^7/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 [71]	Heart failure	15	57.33 ± 10.05	IV	1×10^6 cells/kg	1	Significant improvements in LVEF, NYHA functional class, Minnesota Living with Heart Failure Questionnaire	No
Liver								
Xue et al. [72]	Decompensated liver cirrhosis	50	Unknown	Intrahepatic artery	3×10^7/body	1	Increase of serum albumin	No
Wang et al. [73]	Primary biliary cirrhosis	7	49 (33–58)	IV	5×10^5/kg at 4 weeks interval	3	ALP and γ-GTP	No
Shi et al. [74]	Prevention of acute liver allograft rejection	14 (13, single dose, 1 multiple dose)	57 ± 12	IV	1×10^6 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. [75]	Liver cirrhosis caused by autoimmune diseases	23 (2 CB-MSC, 1 BM MSC)	53.4 (35–70)	IV	1×10^6 cells/kg	1	Not statistically significant improvement	2, fever; 3, mild fidgetiness, suffered from insomnia
Zhang et al. [76]	Ischemic-type biliary lesions following liver transplantation	12	47.3 ± 10.1	IV	1×10^6 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. [77]	Hepatitis B virus-related acute-on-chronic liver failure	30:UC-MSC	UC-MSC: 40.67 ± 9.89	IV	10^5 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		
Gastrointestinal tract								
Zhang et al. [78]	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. [79]	Ulcerative colitis	34	42.9 ± 23.1	IV then IA	0.5×10^6 cells/kg	2, 7 days	Decreases of median Mayo score, histology score. Improvement of IBDQ scores	No
Skin								
Hashemi et al. [80]	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. [81]	Prevention of delayed graft function and acute rejection in renal transplantation	21	41.0 ± 11.5	IV	2×10^6 cells/kg (before transplantation), 5×10^6 (during surgery)	←	No significant improvement	No
Deng et al. [82]	Lupus nephritis	12 MSC, 6 placebo	29 ± 10	IV	1×10^8 cells	2 times 1 wk. interval	Not statistically significant improvement	1: leucopenia, pneumonia, subcutaneous abscess, 1: severe pneumonia
Autoimmune diseases								
Wang et al. [83]	Active and refractory SLE	40	17–54	IV	1×10^6 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. [84]	RA	136	46.1	IV	4×10^7 cells, 2nd in 3 months later	1 (n = 112) 2 (n = 24)	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. [85]	Multiple sclerosis	20	41.2 (24–55)	IV	2×10^7 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
Others								
He et al. [86]	Severe sepsis	15 (3 cohorts)	56 (25–72)	IV	1×10^6 cells/kg	1	System clinical outcomes are not changed	No
					2×10^6 cells/kg			
					3×10^6 cells/kg			
Cao et al. [87]	Recurrent intrauterine adhesions	27	35.1 ± 3.8 (27–42)	Loaded onto a collagen scaffold	1×10^7	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; ES, 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 [88], 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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