

From banked human cord blood to induced pluripotent stem cells: New opportunities and promise in induced pluripotent stem cell banking (Review)

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Abstract. Umbilical cord blood (CB) is a valuable source of haematopoietic stem/progenitor cells (HSCs) and is known for the therapeutic use of these cells in treating blood disorders. However, challenges such as a high running cost and the increasing availability of treatment alternatives have made the effort to sustain CB banks difficult. This prompts the need to revisit the current CB banking initiatives to retain the relevance in this ever-changing era parallel to the fast-pacing development of cell-based therapeutic technology. Cellular reprogramming has shown to have successfully converted adult somatic cells into human induced pluripotent stem cells (hiPSCs), which promise wider applications in regenerative medicine, personalized treatment and tissue engineering. CB is the youngest, primitive adult cell source that has not been affected by any prior, acquired disorders. Hence, using CB as a source of candidate cells for generating hiPSCs may be a new opportunity for banking, albeit with challenges. The present review summarizes the rise and fall of CB usage and banking for clinical therapy, the considerations in reprogramming CB into hiPSCs, the safety concerns regarding the use of hiPSC-derived cells in clinical transplantation and the prospect of using CB-derived hiPSCs.

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1. Introduction

Cord blood (CB) has been a significant source of haematopoietic stem cells. The therapeutic value of CB has been examined since the late 90s when the first transplantation effectively treated a 5-year-old patient with Fanconi anaemia (1). Considering the potential of CB and its viability for autologous use, the practice of CB banking has been extensively embraced to maintain the CB unit from birth, either through government-funded organisations or private companies with a storage charge (2). However, such an approach has also been affected by the high running cost, the actual clinical utilisation and new emergent alternatives (3), thus prompting the cell banking endeavour and its sustainability to be carefully revisited.

Strategies have been introduced to keep the cell banking endeavour relevant, including the introduction of criteria for CB cryopreservation, CB cell expansion, use in allogeneic transplantation and exploring novel medicinal uses. However, the use of CB in the clinic is predominantly focused on treating haematological diseases, which hinders the progress of cell banking initiatives aimed at achieving broader storage and clinical benefits.

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There is an increasing interest in converting CB into hiPSCs as an approach to resolve the underutilized CB unit in the cell bank (4,5), since hiPSCs present with greater therapeutic value and applications. This could potentially enhance the utility of CB and satisfy the rising need for hiPSCs, which could redefine the future of regenerative medicine.

The present review discusses the rise and fall of CB usage and banking for clinical transplantation, including the early discovery of CB characteristics and therapeutic value, the development of past trials that subsequently promoted the growth of the CB bank and industry, the challenge of using low total nucleated cells (TNCs) in CB and the strategy to expand the cells for autologous transplantation, the change in trends of application from intended autologous to allogeneic use, and lastly, the shift of preference to haploidentical haematopoietic stem cell (HSC) transplantation from using allogeneic CB. The review also summarizes the considerations in reprogramming CB into hiPSCs, including the reprogramming methods, the advantages of using CB as the choice of somatic cells for reprogramming, as well as the disadvantages, and the latest strategies for overcoming iPSC allogenicity. Lastly, the prospect of using CB-iPSC banking and the opportunity that it creates is discussed.

2. Rise and fall of CB usage and banking for clinical transplantation

Characteristics of CB and its therapeutic uses. HSCs, which can differentiate into any type of blood cell, are found in umbilical CB. Just 0.02-1.43% of the mononucleated cells in CB are CD34⁺ HSCs, which is a density that is higher than that found in the peripheral blood (PB) (<0.01%) but lower than that found in the bone marrow (0.5-5.0%) (6). The colony-forming cell content has not only been found to be associated with the CD34⁺ cell number, but is also associated with the gestational age of the newborn (7). CB-derived HSCs exhibit a greater growth response to known mitogens and cytokines, and are less reliant on the support of stromal cells (8). These properties allow CB-HSCs to exit the G₀/G₁ phase and divide more rapidly than HSCs from other adult tissues under the same culture conditions (9).

Cord blood (CB) is a youthful and relatively 'disease-free' source of HSCs (10,11), presenting a lower risk of pathogen transmission compared with other adult cell sources (12,13). Comparing HSC transplantation from other allogenic sources, CB offers a lower incidence of acute and chronic graft-vs.-host disease (GvHD), due to the low presence of the alloreactive T cells in the CB unit (14) and the altered cytokine profile (15). Furthermore, CB also offers better tolerance of a greater human leukocyte antigen (HLA) mismatch between the donor and recipient, although the rejection rate increases with the increasing HLA disparity (16). With the increased availability and accessibility of CB banking facilities, transplantation of CD34-enriched CB or multiple partially matched HLA units has become a more viable option for a wider range of patient populations (17).

The first CB transplantation to a 5-year-old patient with severe Fanconi anaemia was performed in 1988 (1), establishing precedence for subsequent use of CB in transplantation between identical siblings after cryopreservation (18).

Complete haematopoietic reconstitution has also been shown to be possible following CB transplantation in HLA mismatched siblings or unrelated patients with leukaemia, as documented in previous studies (18,19). Broxmeyer *et al* (20) reported that nucleated HSCs were found to remain effective and functional even after being frozen for 23 years. CD34⁺ cells isolated from cryopreserved CB were also capable of successful integration and functioning after 6 months in primary and secondary immunodeficient mice, suggesting that cryopreservation does not affect the function of injected HSCs in the long term (20). The growing banking service for CB worldwide has garnered a substantial number of transplantable units for use in clinical transplantation.

According to the World Marrow Donor Association, the global inventory of CB has grown by >800,000 units in 2022 (21), and >40,000 CB transplantations have been performed in both paediatric and adult patients (9), for treating either malignant or non-malignant haematological diseases. In leukemic paediatric patients, the transplantation of complete HLA-matched CB has shown to have comparable effects with bone marrow from sibling donors (22). In children with haematopoietic malignancies, related HLA-identical CB transplantation achieved neutrophil recovery in 90% in patients with a median age of 5 years, and the incidence of GvHD was only 10-12% at 2 years post-transplant (23), with non-relapse mortality at 9% and relapse at 47% at 5 years post-transplant. It was also highlighted that transplanted CB units with higher TNCs contributed to greater neutrophil recovery and a greater disease-free survival rate. Similarly, 2-loci mismatched, unrelated CB units also demonstrated similar therapeutic effects compared with matched adult marrow transplants (24,25).

It was also found that the higher the HLA disparity and mismatch, the lower the 5-year survival rate observed in paediatric donor-recipient pairs who received single unit CB graft (26). The relative risk of CB transplant-related mortality has shown to rise with increasing HLA mismatch (26), despite the lower rate of relapse (25,27,28). This evidence collectively suggests that CB transplantation is the ideal option in paediatric patients provided that the CB unit is sufficiently HLA-matched and the therapeutic cell number is adequate. In conclusion, successful transplantation, especially in pediatric patients, relies on sufficient cell numbers in CB units. Higher TNCs improve outcomes, while proper HLA matching and cell quantity reduce GvHD. Even mismatched CB units can perform similarly to matched adult transplants, highlighting the importance of stem cell numbers for engraftment and survival.

Challenges in CB banking: Usage and cell number. CB from an allogenic source remains the most used in clinical transplantation compared with CB from an autologous source (29). In a survey by the European Society for Blood and Marrow Transplantation, it was shown that, of the 119 patients transfused with CB, 75% of the transplanted grafts were from unrelated donors. As patients are unrelated, HLA matching between the donor CB unit and the recipient patient is important to minimize graft rejection and increase the eventual patient survival time (29). Based on the National Marrow Donor Program and The Centre for International Blood and Marrow Transplant Research (CIBMTR) guidelines, patients and donor CB

Table I. Important parameters determining the therapeutic effect and graft quality of cord blood units.

Parameter and indication	Requirement(s)	(Refs.)
Minimum TNCs for single unit infusion	2.5x10 ⁷ cells/kg (US) >3.0x10 ⁷ /kg (UK and Europe) 2.0x10 ⁷ /kg (Japan)	(30,34,35)
Minimum TNCs for double unit infusion	1.5x10 ⁷ /kg each CB unit	(30,34,35)
Minimum TNCs in the presence of high-degree HLA disparity	2.0-3.0x10 ⁷ cells/kg	(30,34,35)
Minimum TNCs for non-malignant diseases and considering a 4/6 HLA-match	≥4.0-5.0x10 ⁷ cells/kg	(34)
Minimum pre-freezing TNC content	2.5-3.0x10 ⁷ cells/kg	(30,36)
Minimum pre-freezing CD34 ⁺ cells for single unit infusion	≥1.5x10 ⁵ cells/kg	(30,34,35)
Minimum pre-freezing CD34 ⁺ cells for double unit infusion	≥1x10 ⁵ cells/kg per unit	(30,34,35)
Minimum TNCs in collected units based on storage efficiency and cost	0.9x10 ⁹	(37)

HLA, human leukocyte antigen; TNC, total nucleated cell.

grafts should be HLA typed for HLA-A, HLA-B, HLA-C and HLA-DRB1, as well as the confirmatory typing for DQB1 and DPB1, at high resolution using DNA methods (30). However, the availability of such fully HLA-matched CB units (6/6 loci) is only ~10%, and is highly dependent on the race and ethnic group of the CB donor (31). Mismatched CB units at one or two HLA loci could be considered and have been previously found to work in all patients <20 years old and in 75% of patients ≥20 years old (31-33).

Another factor that determines the outcome of the transplantation is the number of TNCs in CB unit. Table I (30,34-37) summarizes the minimum cell number required for single- or double-unit infusion. The Center for International Blood and Marrow Transplant Research (CIBMTR), Eurocord and the European Group for Blood and Marrow Transplantation collaborative group recommended a 2x10⁷ TNCs/kg of recipient for a single unit, and this was found to be associated with sufficient engraftment of progenitor cells and successful transplantation (27). A TNC number <2x10⁷ TNCs/kg proved to have a lower therapeutic value, including delayed engraftment and lower transplant-related mortality (38). Furthermore, a high TNC number (>5.0x10⁷/kg) could subdue the effect of two HLA mismatches, and HLA matching can compensate for the disadvantage of a low TNC number (39). More notably, improved event-free survival rate was found to correlate with higher CD34⁺ cells in the CB unit but was not associated with TNC number (19).

Considering the pros and cons, recommendations are made to ensure the minimum level of both the TNCs and CD34⁺ cells in each cryopreserved CB unit are met (Table I) in order to balance the cost and the therapeutic benefit. Collected CB units that fail to meet these requirements are discarded. These 'unqualified' CB units account for ~36-39% of the total collected CB units (40). This practice not only incurs substantial operational costs involving steps from CB collection to cell analysis, but also involves sacrificing CB units of high therapeutic or research value, especially from donors with rare blood types or diseases (41). Hence, to address low TNC numbers in CB units, the logical and immediate solutions to increase the cell number for transplantation are as

follows: i) Combining two CB grafts (42,43); and ii) expanding the autologous cells to a therapeutically meaningful number *in vitro*.

Ex vivo expansion of CB units as a strategy to increase autologous use in transplantation. A systematic review and meta-analysis of 9 controlled clinical trials (phase I-III) involving 1,146 patients who underwent umbilical CB transplantation (UCBT) (44-52) using *ex vivo* expanded or unmanipulated grafts was conducted by Saiyin *et al* (2023) (53). The expansion strategies employed in the included studies consisted of cytokine cocktails combined with small molecules such as UM171, nicotinamide (NiCord), copper chelation, Notch ligand or Stem regenin-1 (SR-1) (Table II) (49,50,52,54-56) or coculture with mesenchymal stromal cells or double unit transplantation. The meta-analysis concluded that transplantation using *ex vivo* expanded CB significantly reduced neutrophil recovery time in patients compared to transplantation with unmanipulated units. Notably, the infused cell dose did not correlate with time to neutrophil and platelet recovery. Additionally, patients receiving expanded CB transplants exhibited a significant decrease in the risk of death at the study endpoint, and graft failure only occurred in 0 to 10% of patients using expanded cells. Moreover, with the transplantation of expanded CB units using mesenchymal stromal cells or Notch ligand-expanded cells in combination with a second unmanipulated CB, the expanded population were found to be overtaken by unmanipulated CB at 1-year post-transplantation. SR-1 and NiCord expanded CB units achieved graft dominance after a year if double units were used in the transplantation, but this was only seen in 35 and 78% of the patients, respectively (49,51). Nonetheless, the overall CB expansion and time to initial neutrophil recovery do not always improve survival, and there is no statistical difference in the risk of acute GvHD between patients administered *ex vivo* expanded CB units and controls (57). A study has also found that excellent overall survival rate was observed in patients with unmanipulated CB despite the low medium infusion of TNCs and the slower engraftment (45).

Table II. Functions of all tested mitogenic small molecules, factors and cells for cord blood expansion.

Mitogen/strategy for HSC expansion	Functions	(Refs.)
Stem regenin (SR-1)	Selective reversible Antagonist of aryl hydrocarbon receptor that can increase CD34 ⁺ cell number by 330-fold	(49)
UM-171	Small molecule that acts as a CD34 haematopoietic stem cell self-renewal agonist	(54)
NiCord (Omidubice)	Nicotinamide, a form of vitamin B3 and a sirtuin 1 inhibitor that inhibits haematopoietic stem cell differentiation and stimulates proliferation, with improved bone marrow homing and engraftment	(55)
Notch ligand	Activation of Notch receptor induces a 100-fold increase in CD34 ⁺ cells	(52)
Copper chelator	Tetraethylenepentamine (StemEx) inhibits haematopoietic stem cell differentiation and induces the expansion of early progenitors	(56)
MSCs	Haploidentical donor MSCs cultured in serum-free medium supplemented with SCF, thrombopoietin, Flt3 ligand and G-CSF induce a 12.2-times increase in TNCs, a 30.1-times increase in CD34 ⁺ cells and a 17.5-times increase in CFU-Cs.	(50)

SCF, stem cell factor; TNC, total nucleated cell; CFU-C, colony-forming unit cell; G-CSF, granulocyte-colony-stimulating factor; MSC, mesenchymal stromal cell.

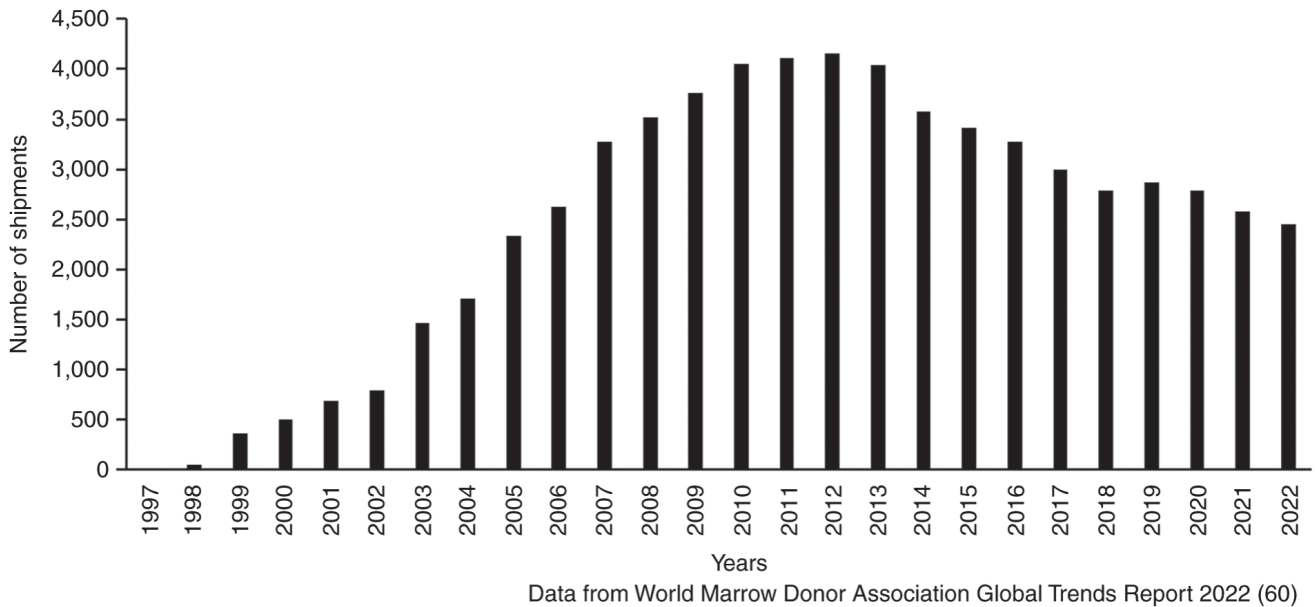


Figure 1. Usage of cryopreserved cord blood units worldwide based on the shipped units under the World Marrow Donor Association between 1997 and 2022.

Decreased utilization of CB: Rise of haploidentical HSC transplantation. The growing banking service for CB worldwide has garnered substantial transplantable units for use in clinical transplantation. Despite the promising growth in the global inventory of CB (21), the total number of units dispensed has reduced from 4,150 units in 2012 to 2,450 units in 2022, a reduction of 41.0% in the past decade (Fig. 1). According to a special report by Passweg *et al* studying the trends of haematopoietic stem cell transplantation in Europe in 2013, CB was only used in 2 out of 22,998 autologous transplantations, while only 5 out of 7,624 allogeneic transplantations involved related family members and 666 out of 8,587 were transplanted to unrelated patients (58). The decline in using CB is also due to the marked increase

in haploidentical transplantation, and a similar trend was also observed by the Asia Pacific Blood and Marrow Transplantation Group (59) and the Worldwide Network of Blood and Marrow Transplantation (60). Allogeneic stem cell transplantation has become the treatment of choice for high-risk haematological malignancies, and the established sources of HSCs for these patients are either CB or haploidentical donors when there is a lack of HLA-matched donors.

Haploidentical donors are immediate family members who possess tissue with half matching HLA haplotypes. The shift of preference towards haploidentical HSC transplantation, particularly from PB, is mainly due to the high cell source availability, fresh HSC isolation from donors upon demand without the need for cryopreservation, a

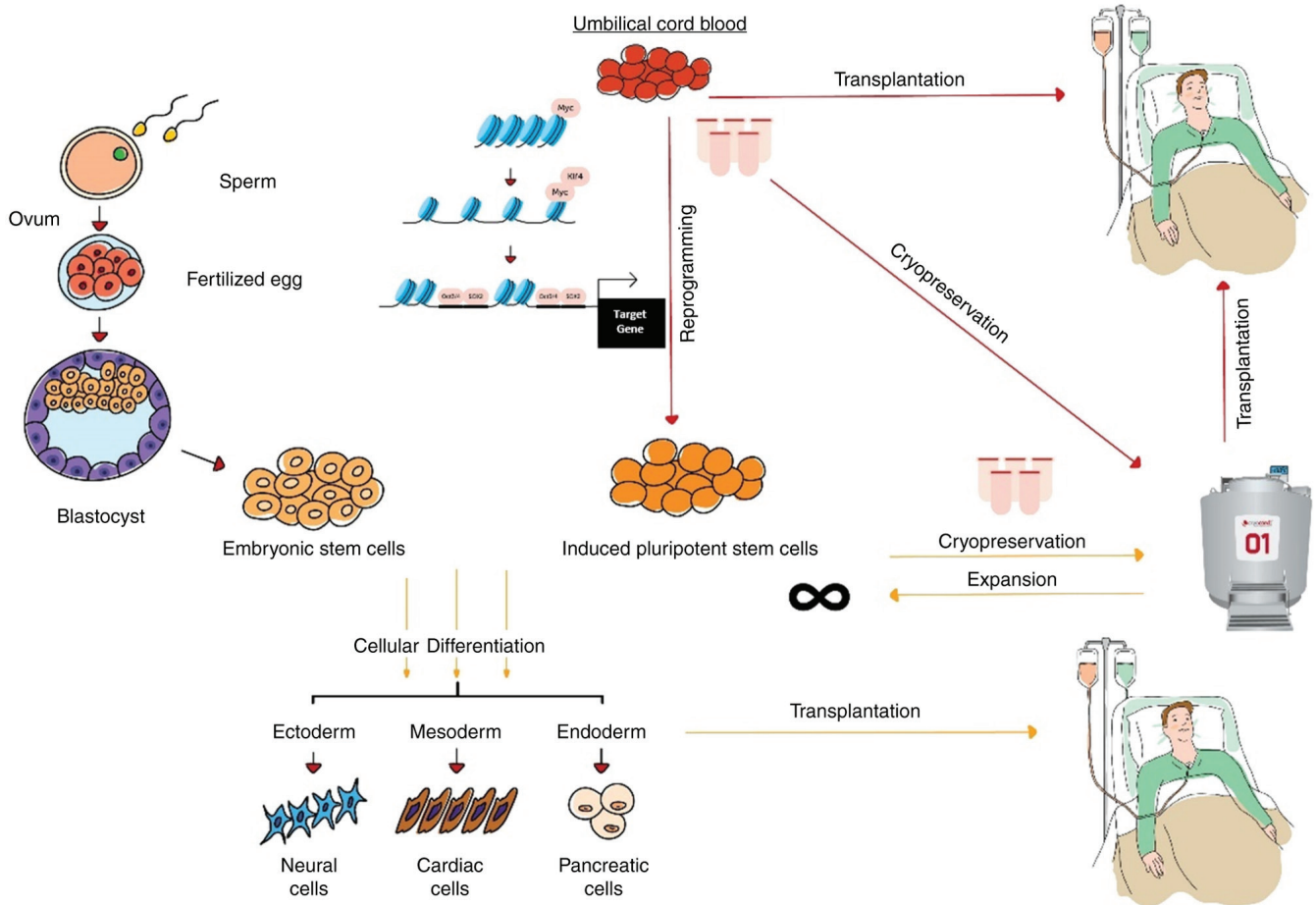


Figure 2. Reprogramming cryopreserved cord blood cells to generate induced pluripotent stem cells.

shorter time to transplantation without the need to mobilize or transport matched grafts from cell banks, such as CB, and therefore a significantly lower cost per transplantation. More importantly, there are no statistical differences in the main outcomes between haplo-HSC and CB transplantation, including relapse incidence, leukaemia-free survival, 2-year non-relapse mortality rate and overall survival rate (61,62). In some trials, CB transplantation demonstrated more disadvantages than the haplo-graft transplantation, including slow neutrophil recovery and a relative higher risk of grade II-IV acute GvHD (63,64).

3. Considerations in reprogramming CB into iPSCs

hiPSCs are cells that are created through cellular reprogramming, a technology introduced by Takahashi and Yamanaka (2006) to reverse the fate of differentiated cells back to the pluripotent stage (65). The invention enables the making of PSCs from any adult somatic cells possible, and the cells possess the differentiation plasticity of the embryonic stem cells. Recently, the induced PSCs were also found to be capable of cultivating synthetic embryos (66), opening vast opportunities to study complex embryology and early human development. Judging from the enormous variety of applications and the therapeutic potential that hiPSCs could offer, and the recent decline in CB transplantation in clinics, several reports have proposed to convert cryopreserved CB

into hiPSCs (4,67) (Fig. 2). Efforts to revamp the cell banking industry and revolutionize regenerative medicine have begun by promoting the use of hiPSCs. The present review discusses and summarizes the information, including the reprogramming methods assessed on CB, the comparison between the use of CB and adult PB, and the advances in establishing hiPSC haplobank or hypoimmunogenic lines as the off-the-shelf cell source for clinical treatment.

Methods of cellular reprogramming to generate iPSCs from CB. Several reprogramming and hiPSC generation methods have been introduced since its discovery. In general, both integrating and non-integrating methods have been used in reprogramming CB (Table III) (20,68-79). Evidence has shown that the reprogramming of CB is possible even it has been frozen for 21 years (20). The outcome of this was also confirmed to be comparable to that of fresh CB (70). The long-term cryopreservation of CB did not impede the formation of embryoid bodies in both *in vivo* and *ex vivo* studies, and the injection of hiPSCs into testis capsules of immune-deficient mice demonstrating spontaneous differentiation into cells of all three germ layers (20). The present review summarizes and emphasizes the reprogramming methods that have previously successfully generated hiPSCs, namely use of retroviruses and lentiviruses, episomal transfection of vectors, and use of Sendai virus, self-replicative RNA (srRNA), microRNA (miRNA/miR) or defined chemicals (Table III).

Table III. Reprogramming methods used in iPSC generation from CB.

Delivery methods	Cell source	Reprogramming factors	CB volume, ml	Time period	Reprogramming efficiency, %	Total iPSC colonies/initial somatic cell number	iPSC colony/ml of blood	(Refs.)
Retrovirus	CB CD34 ⁺ cells	OSKM	NA	~16 days	NA	NA	NA	(68)
	CB CD34 ⁺ cells	OSKM Shp53 pLKO.1-puro	NA	~30 days	0.05	96 colonies/10 ⁴ cells	NA	(69)
Lentiviral	CB endothelial cells	OSNL	NA	~30 days	0.01-0.03	104 colonies/10 ⁶ cells	NA	(70)
	CB CD34 ⁺ cells	OSKM	NA	~19 days	0.03-0.05	NA	NA	(20)
	CB CD34 ⁺ cells	OSKM	NA	~25 days	0.01-1.0	NA	NA	(71)
Episomal vectors	CB CD133 ⁺ cells	OS	8.75	~20 days	NA	0.45±0.27 colonies/10 ⁴	NA	(72)
Episomal vector EBNA1	CB MNC	OSKML	NA	~14 days	0.002	1,000 colonies/2x10 ⁶ cells	NA	(73)
Sendai virus vector	CB CD34 ⁺ cells	OSKM	5	~28 days	0.01	NA	10	(74)
Sendai virus	CB CD133 ⁺ cells	OSKM	NA	~21 days	0.02-1.0	NA	NA	(75)
Sendai virus vector-free srRNA	CB erythroid progenitor cells	OSKM	NA	~17 days	0.03-0.19	NA	NA	(76)
mRNA cocktails	UCB MSC	OSKM	NA	~30 days	0.8-1	~27 colonies/10 ⁵ cells	NA	(77, 78)
		OSKM and GLIS1	NA	~25 days	NA	NA	NA	(79)

iPSC, induced pluripotent stem cell; CB, cord blood; sRNA, self-replicative RNA; MSC, mesenchymal stromal cell; MNCs, mononuclear cells; UCB, umbilical cord blood; NA, not applicable; OSKM, OCT3/4, SOX2, KLF4 and MYC; OS, OCT3/4 and SOX2; GLIS1, GLIS family zinc finger 1.

Retroviruses. Retroviruses rely on the infectivity of host cells via receptor-mediated endocytosis to achieve stable genomic integration of the RNA reprogramming factors with the aid of the reverse transcriptase and integrase enzymes (80). The transgenes transduced into host cells allow for stable expression and maintenance for 30 days before generating the iPSCs. CD34⁺ cells that were transduced with retroviruses carrying Yamanaka's factors and p53 short-hairpin RNA vectors enhanced the reprogramming efficiency. p53 short hairpin RNA vectors are crucial in inhibiting the p53 pathway (69). However, retroviruses are prone to insertional mutagenesis, raising the safety concern over their use in the clinic (68).

Lentiviruses. Lentiviruses are RNA viruses that are capable of infecting both dividing and non-dividing cells. After reverse transcription, the lentiviral genome will be integrated into the host cell genome, allowing long-term expression of the inserted genes in the target cells *in vitro*. Haase *et al* (70) successfully generated iPSCs from proliferative CB endothelial cells and yielded better results than with PB. Lentiviral vector-mediated transduction of *OCT3/4* and *SOX2* alone was found to sufficiently reprogram CB CD34⁺ cells into hiPSCs with an efficiency of 2% (81), an outcome that was higher by 1,000-fold than that of using a retrovirus.

However, early lentivirus vectors tend to integrate transgenes into target cells, leading to host genome sequence changes (81). These transgenes were not silenced in the generated hiPSCs and could result in tumour formation, especially due to the high expression of *c-Myc* and *Klf4*, known oncogenes. A safer, self-inactivating third-generation lentiviral system has been introduced, with the packaging system containing two plasmids: the Rev encoding and Gag and Pol encoding plasmids. Multiple clinical trials have shown that third-generation lentiviral vectors can introduce genes to HSCs to treat hemoglobinopathies and primary immunodeficiencies (82,83). However, the use of third-generation lentiviral systems is still not applied in iPSC generation.

Episomal vectors. The non-integrating, self-replicating vector delivery system using EBNA1/OriP, capable of long-term persistence in cells to mediate nuclear import and retention of vector DNA, allows hiPSC derivation from a single transfection. EBNA1 vectors express the EBNA1 gene to deliver episomal vectors into somatic cells. This method is a transgene-free approach, whereby episomal vectors are removed from iPSCs without integration into genomic DNA (72). A study using CB, which has been cryopreserved for 13 years, demonstrated that transduction with EBNA1/OriP resulted in a highly efficient generation of hiPSCs obtained

within 14 days, with 1,000 hiPSC colonies created per 2 million transfected cells. A parallel experiment with PB-mononuclear cells indicated a 50-fold lower efficiency in deriving hiPSCs compared with that for CB (73).

Sendai virus. Also, the non-integrating method using the Sendai virus vector (SeV) can generate hiPSCs without foreign gene insertions into the host genome. This approach is highly applicable for future clinical use, as SeV can be produced with temperature-sensitive mutations, making the vectors easily removable at non-permissive temperatures, thereby eliminating the foreign genes in the mature hiPSCs (74). Another study demonstrated that CD34⁺ CB could be reprogrammed with volumes as low as 5 ml for each procedure using a viral mixture of SeV TS7-*OCT3/4*, *-SOX2*, *-KLF4* and *-c-MYC* to generate 10 independent hiPSCs per ml of blood (74). In 2019, the SeV reprogramming method was used to reprogram the CB CD34⁺ cells of a female child to create iPSCs (75). Recently, Kunitomi *et al* (84) developed a more precise and versatile SeV-KLF4 by modifying the vector to include L-MYC in place of c-MYC, and also adjusted the temperature settings during cell maintenance and reprogramming. Thus, naive hiPSCs showed a significantly improved ability to differentiate into trilineage lineages (85).

srRNA and miRNA. Reprogramming can also be achieved by using srRNA or miRNA. The non-integrating srRNA that encodes Venezuelan equine encephalitis (VEE) replicon carried the four reprogramming transcription factors OCT3/4, SOX2, KLF4 and cMYC, or with GLIS1 in replacement of cMYC, was found to successfully induce reprogramming in newborn or adult fibroblasts (86). This reprogramming process was facilitated by supplementing B18R recombinant protein, an essential virus-encoded receptor, which increased cell viability during RNA transfection, that neutralizes type 1 interferon induced by VEE RNA (86). In addition, the ES cell-specific cell cycle-regulating miRNAs miR-291-3p, miR-294 and miR-295 also showed enhanced retrovirus-mediated expression of *OCT3/4*, *SOX2* and *KLF4* (87). Similar mRNA-facilitated srRNA reprogramming was also employed and assessed in a study that compared the reprogramming efficiency of CB-derived endothelial progenitor cells (CB-EPCs) with PB-EPCs. CB-EPCs were found to be more efficiently reprogrammed than PB-EPCs, as shown by a 3.5-fold increase in colony formation and cell number, along with results from pluripotent gene expression microarrays (77).

Chemical approach. An alternative reprogramming method has been introduced using small molecules to replace the conventional gene-mediated reprogramming method. In the study by Chou *et al* (73), it was demonstrated that the use of the small molecule compound VC6TFZ, consisting of 6 specific molecules [valproic acid sodium salt (VPA), CHIR99021, 616452, tranylcypromine, forskolin and 3-deazaneplanocin A (DZNep)], followed by dual inhibition of glycogen synthase kinase and mitogen-activated protein kinase, could efficiently generate hiPSCs from mouse embryonic fibroblasts without the ectopic expression of reprogramming genes (88). Nonetheless, this method has not been used in reprogramming CB cells into hiPSCs, although direct programming of CB erythroblasts to induced megakaryocytes was demonstrated using Bix01294, PD0325901, VPA and RG108 (89).

Factors that affect reprogramming and the subsequent iPSC differentiation and quality. The cellular reprogramming process involves resetting the epigenetic landscape of a somatic cell and reverting it back to a pluripotent state. Studies have shown that cellular reprogramming and the subsequent differentiation efficiency are affected by several factors, which include the age of the parental somatic cells, cell origin, type of cell and the method of reprogramming or differentiation (90-93). The types of parental somatic cells used in reprogramming do not seem to have induced variability in the resultant hiPSCs. For instance, a study that tested fibroblast and blood cells from the same donor showed no major variability between the produced hiPSCs, in terms of both reprogramming and differentiation efficiency (94), but evidence has proven that the retained residual epigenetic memory in the generated iPSCs could make the cells prone to differentiation towards their tissue of origin (91,92). Moreover, hiPSCs derived from cells of genetically different donors revealed significant differences in the hiPSC differentiation efficiency (90,94).

Additionally, higher reprogramming efficiency was observed when younger, juvenescent somatic cells that possess less epigenetic modifications were used (95). A study by Lo Sardo *et al* (95) showed that hiPSCs generated from the PB of young individuals in their twenties, middle-aged individuals at 49-79 years old and the elderly at 86-100 years old showed no difference in reprogramming efficiency. The study also reported that a significantly higher level of methylation was found in donor cells from older individuals, with a 5% increase in the global methylation level at CpG sites (95). This makes the relative resistance to demethylation in age-associated CpG sites higher during cellular reprogramming in older donors. This is in line with the observation reported in the study by Gao *et al* (2017) (77), which compared hiPSCs derived from EPCs, CB and adult PB. The study showed that a higher reprogramming efficiency was evident in CB-EPCs compared with that in PB-derived cells. Furthermore, hiPSCs from aged donors retained an epigenetic signature of age, which could be slowly reduced through *in vitro* passaging, but the older the parental cells, the higher the risk of exomic mutation present in the generated hiPSCs, as evidenced by whole-exome sequencing analyses that demonstrated an increased frequency of mutations correlating with donor age (95).

Advantages and disadvantages of CB as the somatic cells of choice for generating hiPSCs. The most discussed advantages of using CB as the cell source for generating hiPSCs are the availability of the cells in CB banks, the primitive nature of the cells (free from any acquired mutations such as those found in other somatic cells) and their young age. Studies have shown that the function of the generated hiPSCs does not seem to be affected by the age of the donor cells (96), as the epigenetic signature of ageing in hiPSCs can be reduced with *in vitro* passaging (95). In general, CB-derived cells were more proliferative than those from adult PB (70), and the advantage of using youthful CB cells was evident in their reprogramming efficiency (95). However, no difference in differentiation was found when comparing the hiPSCs from CB and PB (73). Other studies have also concluded that the differentiated cells from different hiPSCs donor sources possess no difference, or functional superiority in *in vivo* heart function after transplantation (92).

Nonetheless, a significant drawback in generating hiPSCs from CB is the additional cost required for cell separation to purify mononuclear cells or CD34-expressing HSCs, similar to the situation in PB. This process is necessary, as attempts to reprogram red blood cells into hiPSCs have not been successful due to the lack of a nucleus in mature red blood cells.

4. CB-hiPSC banking for allogeneic transplantation

Graft immunogenicity and HLA mapping. The use of CB-iPSCs only for autologous means may incur a substantial cost of preparation and maintenance, and it is more applicable to personalized and precision testing, and treatment in patients with specific conditions or family members. Allogeneic use of the cells is, however, more likely to be the option to reduce the cost of maintenance and to maximize the usage across different patients. One of the strategies to lower the immunogenicity of CB-hiPSC-derived cells is to identify HLA haplotype homozygous CB donors. Research has shown that cells from HLA haplotype homozygous donors can benefit significant numbers of patients in the country or populations with low HLA diversity (97,98). By using this method, off-the-shelf allogeneic CB-hiPSCs that are haploidentical to a good majority of the population can be generated in large quantity and decrease the time and cost when compared with individualized hiPSC manufacturing.

In Japan, a clinical-grade hiPSC haplobank was established with 27 hiPSC lines derived from 7 HLA-homozygous donors, which was claimed capable of covering 40% of the Japanese population (99). The haplobank has provided the iPSCs for more than 10 clinical trials since 2015. Similarly, in Spain, an iPSC haplobank was established through a search of 32,000 bone marrow donors and CB donors from the Spanish Bone Marrow Donor Registry (99). A total of 10 CB units from homozygous donors stored were found to meet HLA-A, HLA-B and HLA-DRB1 matching for 28.23% of the population (100). The efforts also led to the formation of The Global Alliance for iPSC Therapies, which consolidate and assess the feasibility in developing the global haplobank partnership in the generation of clinical grade hiPSCs (101).

Hypoimmunogenic hiPSC line. Autologous cell transplantation is still a favourable option considering its better graft engraftment and low immunogenicity compared with allogeneic cells post-transplantation. However, generating hiPSCs processed from an autologous source can be costly and time-consuming, while long-term immunosuppression may be needed if hiPSCs from an allogeneic source are used, despite some precedence of successful clinical translation (Table III). The major underlying cause of the immune rejection is the highly polymorphic major histocompatibility complex (MHC) molecules, the primary trigger of T-cell-mediated cytotoxic responses upon the recognition of the alloantigen presented on antigen-presenting cells.

Recently, a strategy to remove the MHC class I and II expression, by gene-editing the *B2m* gene (a structural component of MHC class-I molecules) and *Ciita* (the master regulator of MHC class-II molecules), has been introduced to reduce the cell-immunogenicity, cloaking the cells from the immuno-surveillance by T cell-mediated adaptive

responses (102). The cells were also engineered to express CD47 transmembrane protein alone (103) or with immunoregulatory factors inhibitors, such as program death-ligand 1 and human leucocyte antigen-G, to better prevent both the T cell- and natural killer cell-mediated immune responses, as well as macrophage engulfment (104). These methods have successfully created universal, allogeneic hiPSCs with 'hypoimmunogenic', without compromising the cell pluripotency, differentiation and post-differentiation cell functions, as demonstrated in humanized animals (genetically or biologically modified to express human genes or contain human cells, tissues or organs) post-transplantation in the absence of immunosuppression (103).

5. Safety concerns over the use of hiPSC-derived cells in clinical therapy

A critical issue concerning the clinical use of patient-specific hiPSCs is the accumulation of somatic (stem) cell mutations over an organism's lifetime. Acquired somatic mutations are passed on to hiPSCs during reprogramming and may be associated with the loss of cellular functions and cancer formation (105). Cell reprogramming has also been associated with defective epigenetic reversion and gene expression changes, which raise safety concerns and the possibility of treatment failure. hiPSCs would inevitably retain a certain form of epigenetic memory linked to their parental cells (91). The presence of these epigenetic signatures would thus skew the differentiation potential of these cells to their tissue of origin.

Additionally, other studies have determined that modifications related to neoplastic gene methylation and altered genomic imprinting are present in hiPSC lines, which can cause malignant transformation and abnormal differentiation (106,107). Efforts to overcome the genomic instability of hiPSCs centre on implementing two main approaches: Genetic screening and the prevention of aberrations. These measures are crucial in ensuring the accuracy and reproducibility of study outcomes, which at the right time determine the feasibility of translating hiPSCs to clinical applications.

As abnormalities have been found at various levels of the genetic hierarchy, the evaluation of hiPSCs is recommended based on three pillars: Karyotyping for the detection of balanced aberrations, chromosomal microarray for the identification of copy number variants and next generation sequencing for the determination of single nucleotide variants or indels. These genetic assessments are ideally conducted on each hiPSC line before being used for research or therapeutic purposes. In the endeavours to prevent aberrations, recent research has discovered that adding antioxidant supplements to the culture media of hiPSCs can reduce genomic instability (108,109). This has been attributed to the alleviation of the oxidative stress cells are subjected to during the reprogramming and early passage cultures. Furthermore, research using small molecules to reprogram cells indicated that this approach could generate hiPSCs without compromising genomic stability (110). The mechanism of action leading to this observation is believed to stem from initiating the expression of the *Zscan4* gene and the prevention of DNA double-strand breaks (111).

As with the case of reprogramming hiPSCs from CB, this strategy to repurpose CB to hiPSCs is viewed as advantageous to prevent genomic instability, as it can exclude any genetic aberrations accumulated from adult cells, is void of most epigenetic imprinting and retains the cellular age of a newborn, thus preserving telomeric length for greater proliferative capacity. Furthermore, a protocol has been developed to use two reprogramming factors, *OCT3/4* and *SOX2*, to generate CB-hiPSCs (72). A recent study also disclosed that using improved lentiviral vectors with high efficiency (2-14%) achieved enhancements such as optimized promoter design, and incorporated genome stabilizers, such as *Zscan4*, which could reduce reprogramming-induced mutations (112). This method generated CB-iPSCs that harbour an average of only 1.3 coding mutations per cell line, as determined by whole-exome sequencing. These findings significantly improve the generation and manufacturing procedures of hiPSCs, and serve as a step forward to bring hiPSCs and their derivatives to clinical use.

6. Future perspectives of CB-hiPSCs: Banking and applications

One of the most promising prospects is the realization of autologous CB-hiPSCs. This will allow patients to benefit from their own reprogrammed cells, reducing the risk of immune rejection and improving treatment outcomes. Additionally, CB-iPSC lines derived from donors with rare blood groups could pave the way to manufacture blood cells of unique characteristics as differentiation technologies advance, meeting the demand for transfusion in patients in need of rare blood (40). Furthermore, analyzing primitive CB-hiPSCs across different developmental stages may provide valuable insights into commonly acquired mutations, enhancing our understanding of genetic changes in human disease development and aging.

In the US, >400,000 CB units have already been HLA-typed (113), making the starting materials for creating CB-hiPSCs more readily available than other cell sources. This abundance of HLA-typed CB-hiPSCs would create a robust pool of cell lines for selection and matching with recipient patients. The integration of big data and artificial intelligence would further optimize this process, minimizing immunogenic risk. This approach complements the concept of a 'Haplobank', a repository of partially matched CB-iPSC lines usable for most of the targeted population. Together, these strategies would synergistically increase the therapeutic use of CB-hiPSCs with minimal immunogenicity, reduce manufacturing costs and accelerate advancements in complex applications such as organ engineering, collectively making regenerative therapies more affordable and accessible.

7. Conclusions

hiPSC technology offers great promising in regenerative medicine, judging from the pluripotent capability of the cells to differentiate and regenerate tissues, including those that were previously considered impossible to regrow. Reprogramming CB cells into hiPSCs is a viable strategy to expand their use to a wider range of diseases and reinvent the banking business that limits the current growth of CB banking services. The formation of an iPSC haplobank based on the existing

cryopreserved CB donations could serve as a good cell source for regenerative therapy, not only in the context of allogeneic transplantation, but also as the foundation for use in autologous tissue regeneration or organ engineering in future.

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Authors' contributions

FFR, YXY, JJT, SKC, ZG, AP, GCO, KLT, KYT and MNA contributed to the review conception and design, interpretation and writing. FFR, YXY and JJT wrote the first draft. SKC, ZG, AP and JJT reviewed, revised and proofread the manuscript. All authors read and approved the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

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Not applicable.

Competing interests

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