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# Concise Review: Cord Blood Banking, Transplantation and Induced Pluripotent Stem Cell: Success and Opportunities

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ABSTRACT

**Hematopoietic cell transplantation (HCT) has become a standard practice to treat a number of malignant and non-malignant hematologic diseases. Bone marrow, mobilized peripheral blood, and umbilical cord blood can all serve as primary sources of cells for HCT. The number of cord blood units currently stored is large, although it represents only a fraction of potential collections. With much of the collection being sequestered in private banks for possible autologous use, there is a reason to expect that public banks may not be able to provide for the demand in coming years as use of cord blood for treatment of patients with diseases such as leukemia and lymphoma continues to increase. We suggest that a possible solution to encourage private banks to share their valuable units is to apply recent methodologies to generate induced pluripotent stem cells from cord cells and to optimize techniques to generate hematopoietic lineages from them. This strategy would allow us to take advantage of the units already collected under appropriate regulatory guidelines, to access a pristine cell that can be converted to a pluripotent cell at a much higher efficiency and in a shorter time period than other cells. The ability to potentially replenish a used cord unit with new cells, as well as extend the potential utility of cord blood for additional therapeutic applications, should allow banks to develop an appropriate business model for both private and public cord blood banks to flourish.** STEM CELLS *2012;30:55–60*

Disclosure of potential conflicts of interest is found at the end of this article.

## INTRODUCTION

Hematopoietic cell transplantation (HCT), also commonly referred to as bone marrow (BM) transplantation, was first performed successfully 40 years ago [1, 2]. Currently, 50,000 patients per year receive HCT typically to treat malignant diseases such as leukemia, lymphoma, or multiple myeloma [3], and there are now approximately 11 million human leukocyte antigen (HLA)-typed donors in international donor registries [4]. Despite the development of marrow registries, approximately one-third of patients who need an allogeneic HCT are currently unable to find an appropriate "adult" donor match.

Following the successful transplant of cord blood to treat Fanconi anemia in 1989 [5], umbilical cord blood (UCB) has emerged as an alternative rich source of hematopoietic stem cells [6, 7]. This has translated to a now rapidly developing medical field, described in several recent reviews [4, 8–10]. There have been more than 15,000 cord blood transplants worldwide by 2009, and in the United States, more than half of all stem cell transplants from unrelated donors in children now use cord blood (http://www.nationalcordbloodprogram. org).

The use of UCB for HCT provides some potential advantages compared with the use of BM or mobilized peripheral blood (PB). Advantages include prompt availability, decreased risk of transmissible viral infections, reduced incidence of graft-versus-host disease (GVHD), and ease of collection with little to no risk to the mother or newborn [4, 11]. In contrast to BM or PB that generally require a high degree of HLA match between donor and patient [11, 12], UCB only needs to be matched at four of six HLA class I and II molecules. This reduced incidence of GVHD with partially HLA-mismatched UCB is likely due to the lower numbers of T cells and the relatively immunologically naïve status of the lymphocytes in units of UCB [11, 13].

Initial trials using UCB for HCT focused on pediatric patients for two main reasons. One logistic reason was that for the first clinical use of UCB, the donated unit was obtained from an HLA-matched sibling. Sibling donors are preferentially used for BM and PB HCT as complications are fewer and survival is improved compared with the use of unrelated allogeneic donors. Second, pediatric recipients are small, and there are enough hematopoietic stem cells (HSCs) (as measured by $CD34^+$ cells) in a single unit of UCB to engraft in a pediatric patient in acceptable amount of time to prevent complications. In contrast, in adults it took a month or more to engraft and resulted in significant morbidity and mortality [14–16].

To expand the utility of this rich source of HSC, considerable effort has been invested in developing methods to make

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UCB more suitable for adults. One pursuit has been to define conditions for ex vivo expansion of the HSCs in a unit of UCB so that more cells that provide long-term multilineage engraftment can be obtained. Most efforts to support expansion of UCB (or PB or BM) lead to production of hematopoietic progenitor cells that may provide some improved short-term engraftment of myeloid cell lineages [17, 18]. While this is potentially beneficial, studies to more effectively expand true HSCs capable of life-long engraftment remain a priority in hematopoiesis research and constitute an intriguing challenge for UCB stem cell biology [10].

A second approach to improve clinical use of UCB for adults, pioneered at the University of Minnesota, has been the infusion of two units of UCB to one patient. In current clinical studies, patients are given two units that are both at least four of six HLA-matched to the patient and each other. The combined cell dose allows substantially improved time-to-engraftment for adults compared with the use of a single UCB unit and this "double UCB transplants" (DUCBT) has been a remarkable clinical success [4, 15, 19, 20]. A recent clinical study of 536 adult patients with hematologic malignancies at the University of Minnesota and Fred Hutchinson Cancer Center (Seattle, WA) compared results of HCT using DUCBT, HLA-matched related donors, matched unrelated donors, or one-antigen mismatched unrelated donors as cell sources [20]. This analysis demonstrated that leukemia-free survival was similar for patients who received allogeneic cells from cord blood or adult donors. Indeed, risk of relapse was lower in the DUCBT patients.

In parallel to the increasing clinical use of UCB worldwide, an entire industry to collect and store UCB has developed. Two competing models have developed a public cord blood bank model supported by public funds akin to the blood bank and BM programs and a competing private cord banking business where commercial "private" entities offer to store UCB for use by a particular child or family. One recent report tallies 36 public, nonprofit UCB banks in 36 countries and at least 150 private, commercial UCB banks worldwide [21]. For a list of accredited public cord blood facilities worldwide, see http://www.factwebsite.org/ and for the presently most comprehensive list of private cord blood bank sites worldwide, see http://parentsguidecordblood.org/. Despite the growth of the collection industry, total UCB collections represent less that 5% of potentially available cords and increasing donation rates by even a small percentage could significantly increase the number of available units.

## Issues of Private and Public UCB Banking

Registries of potential allogeneic adult donors, as well as UCB units in public banks, have become instrumental to facilitate allogeneic HCT for patients who do not have a suitable HLA-matched related (typically sibling) donor. Considerable societal benefit is garnered from this UCB donation and public banking. Efforts in the United States to increase collection of UCB units have been partially supported by recent legislation such as the Stem Cell Therapeutic & Research Act of 2005. To date, access to UCB has generally not been a significant problem for patients who need this therapy. However, with the success of clinical trials using DUCBT for adults, including the use of reduced intensity conditioning to benefit older patients or those with comorbidities that make fully myeloablative conditioning too risky, the existing system of UCB collection, storage, and distribution could become strained in the future.

Collection of donated UCB by public banks is in direct competition with private banks that have prospered by catering to parents and guardians who may wish to do everything for the future benefit of their children. Private UCB banks aggressively market the collection and storage of UCB to expectant parents, and these individuals have a high motivation to consider the opportunity to store UCB as offered by private cord blood banks rather than donate for the common good. These private banks charge a fee for processing the sample and its subsequent storage. In the process, private cord blood banks spend significantly to educate the public about HCT and establishing relationships with clinics to ensure a supply chain. Private cord blood banks have grown rapidly, although as would be expected collections are restricted to more affluent segments of society.

Indeed, the total number of cord units in private banks far exceeds the number preserved in public banks while the majority of units actually used for therapy come largely from the public banks. This is understandable as on an individual level, the probability of using the stored cord blood unit is relatively low (fortunately for the individual). By various estimates, the chance of an individual receiving his/her own UCB as treatment for one of many hematological disorders where HCT plays a role ranges from probabilities of 1:2,500–1:200,000 [21, 22], a more precise estimate being difficult to discern. This use may be slightly more frequent if donor cells were also available to family members, although it must be noted that a single unit of UCB is not typically sufficient or optimal for adult HCT. Moreover, it is possible that if a child develops a childhood malignancy that could be treated by UCB, there may be malignant cells in the UCB itself rendering the treatment ineffective [21–24]. Based on these calculations, the vast majority of cord blood units stored for autologous use in private UCB banks will not be used and may potentially be wasted. Furthermore, if a potential recipient of a stored autologous unit of UCB has grown to greater than approximately 50 kg, then two units of UCB will likely be needed to ensure prompt engraftment. Therefore, having one stored unit of UCB may be helpful, but this may not be sufficient for HCT.

Differences in the process of UCB collection and the failure to type samples by private cord blood banks make it difficult to search for unrelated HLA-matched donors in private banks or transfer units from private banks to public banks even if one wished to do so. Regulatory guidelines defining processes of transferring a family-bank stored product to a public bank do not exist. Given that samples frozen for autologous use (i.e., private/family banking) are not always comprehensively characterized (e.g., typed for HLA), transfers may prove difficult particularly for the majority of UCB units that are stored in single compartment bags where no sample can be removed for HLA typing or other characterization. Transfers of existing samples to public banks may require further testing to fulfill the proper requirements for nonautologous use. This will add costs for analysis and regulatory compliance and in many instances may not be feasible.

In addition, the recent changes in regulations suggest more regulatory burden than in the past, and there are significant concerns regarding the private banking model [21, 22]. The idea of private blood banking has sparked numerous ethical debates and a number of professional societies worldwide have issued statements/policies that address the conflicting interests between public and private UCB banking (reviewed in ref. [21]. Uniformly, these groups discourage the use of private UCB banking and encourage families to donate UCB to public UCB banks. Many of us in the field contend that

health choices should be egalitarian as has been espoused in the laws governing organ and marrow transplant, where selling organs is illegal. Private cord blood banking is likely to reduce the amount of sample availability in the public banks and appears to be encouraging clinicians involved in the collection process to endorse an unproven therapy. As samples themselves are unlikely to be used, banks have indulged in exaggerated or even false claims to promote the idea of banking. The situation is thus suboptimal from both ethical and commercial standpoints.

Nevertheless, private cord blood banks spur initiative and have to large extent pioneered storage and cryopreservation procedures and the establishment of collection facilities. Proponents point out that they have collected a large repository at considerable expense that may be of value to society and more importantly may provide insurance for people with the means to afford it. As with any private enterprise, free market forces are working to open up this use to an ever larger fraction of the population and make private banking an option for ever more individuals. It also appears that prohibiting parents from donating to private banks is not a reasonable approach.

**Table 1.** Benefits to use UCB for iPSCs

| |
|---|
| 1. Tissue sourcing is well organized and processes are validated. |
| 2. HLA typing data are already being collected by public banks. |
| 3. Existing samples can be used without compromising their ultimate use. |
| 4. A remuneration model already exists. |
| 5. Integration-free methods work well with cord blood cells. |
| 6. A xenofree media and protocol has been developed so clinical-grade iPSC lines can be made. |
| 7. Cord blood is the youngest source of stem cells one may obtain reliably and easily. |
| 8. Stem cells in general have specific mechanisms to maintain genomic integrity, delay senescence, and protect against transformation. |
| 9. The process can be easily extended to blood banks and marrow-derived CD34+ cells using the same infrastructure. |
| 10. Somatic memory and differentiation bias may work in our favor for early therapeutic efforts. |
| 11. May stimulate the cord blood banking business. |

Abbreviations: HLA, human leukocyte antigen; iPSC, induced pluripotent stem cell.

## Induced Pluripotent Stem Cell Derivation From UCB

The ability to reprogram any adult cell using defined factors was pioneered by Yamanaka and colleagues in 2006 [25] and the field has been seen extraordinary rapid progress and numerous novel breakthroughs, as recently reviewed [26–28]. Overall, the large number of independent publications and meta-analysis of the published data suggest that induced pluripotent stem cells (iPSCs) closely resemble ESC derived from blastocysts and that like ESC, iPSCs can contribute to the germline in chimeras in mice and that gene expression profile shows no greater variation than that seen among different ESC lines [29, 30]. Human iPSC as such may be functionally interchangeable with hESCs and like hESCs can generate diverse hematopoietic lineages [31–33]. These findings make human iPSCs potentially useful for novel hematopoietic and immune-based therapies as well as studies of genetic diseases that effect hematopoietic development [34].

The initial derivation of iPSC relied on using retrovirus and lentivirus transformation of cultured fibroblasts derived from patients. Since that initial description there have been many advances that have both reduced the possibility of deleterious effects of integration, persistent expression or reactivation of the inducing genes as well as increasing the efficiency of the induction process. These techniques range from using excisable all-in-one constructs (e.g., CRE_LOX flanked or piggyback or *Sleeping Beauty* transposon-based vectors), episomal vectors (plasmids, minicircles, and episomal viruses such as bacculovirus or sendai), using RNA, protein, or small molecules that activate the specific pathways [27, 29, 30, 35]. Researchers have also examined the cell types that may be the most amenable to induction of pluripotency, and in general, it appears that most cell types can be induced to become pluripotent but the frequency and efficiency depend on the age of the sample (younger is usually better). Cell types that already express some of the pluripotency genes appear to be reprogrammed more efficiently or with fewer exogenous factors being required [27, 29, 30, 35].

Overall, the large number of independent publications and meta-analysis of the published data suggest that iPSCs superficially resemble ESC derived from blastocysts and that like ESC iPSCs can contribute to the germline in chimeras in mice and that gene expression profile shows no greater variation than that seen among different ESC lines. iPSC as such may be functionally interchangeable with ESC even though more subtle differences may be present.

Investigators have suggested that such subtle difference and their consequence may not be detected in standard assays but may have profound consequences on long-term survival or behaviors of iPSC particularly after transplantation. These include issues such as long-term karyotypic stability, rate of accumulation of mutations, ability to maintain telomeric ends and protect against senescence, the activity of imprinted genes, and overall epigenomic profile, mitochondrial integrity and number, and differential immune response in transplantation assays. Despite possible subtle differences between ESC and iPSC, the relative ease with which iPSC can be obtained, the ability to prospectively identify a ideal donor, and the ability to make multiple lines from the same donor all make iPSCs occasionally more useful and often more practical to generate than ESC for many applications.

The availability of well-characterized, HLA-typed cells collected with appropriate consent coupled with recent breakthroughs in iPSC generation suggest that cord blood cells may represent a good source of cells for such an effort (Table 1). Recently, protocols for transformation of cord blood cells to iPSC have been reported by several groups [36–39].

Two important points are worth emphasizing. The efficiency of generating iPSC from cord blood-derived stem cells is not only higher but also faster, as the absolute number of cells that can be obtained from a small fraction of the cord blood aliquot far exceeds what is required for a cell line generation. In addition, if good patient history is available and if consent forms are well written as is common with all blood bank registries it is also possible to collect additional cells or additional data for further follow-up.

On a practical level, it is likely that the residual blood that is present in the tubing at the time of collection contains sufficient amount of cells to be frozen separately so the entire sample need not be thawed for this purpose. Of course, a small aliquot of cord blood could also easily be frozen separately at the time of collection, or iPSC generation could be planned at the time of use of a cord blood unit. These options provide a system to generate iPSCs that can be included

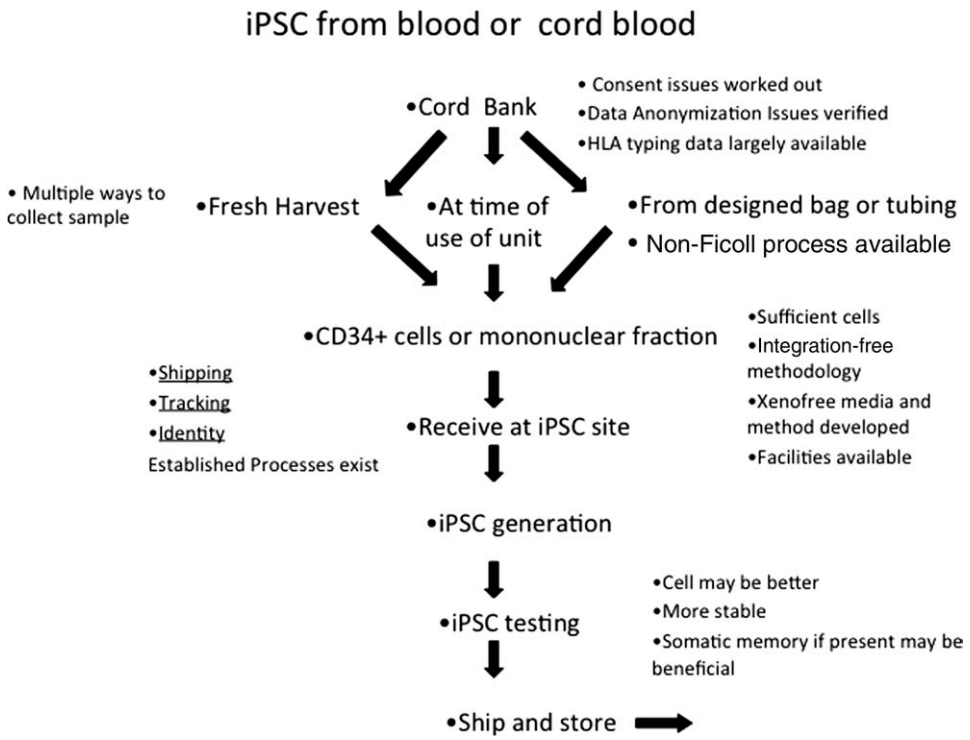


**Figure 1.** A hypothetical process of iPSC generation from cord or marrow cells is diagrammed. Note that cord blood samples for iPSC generation could be collected at different time points and thus would not affect cord blood processing for CD34+ cells. Abbreviations: HLA, human leukocyte antigen; iPSC, induced pluripotent stem cell.

readily in the workflow without any major changes to current processes (see Fig. 1).

iPSC generation also may allow private blood banks to be able to share their samples without compromising on the contracts they have entered into with the donors who pay to have their samples stored for their own use. It may also allow private blood banks to consider additional sources of revenue and increase the use of their stored sample which in turn is likely to increase the number of potential donors willing to store samples. Current penetration rate of cord blood storage is under 5% of total possible collections.

One can imagine a workflow process where cord blood is shipped to a facility, a small aliquot is removed, and iPSC lines are generated at the same site using a zero-footprint process such as plasmids, or minicircles or Sendai virus (each of which can be manufactured using a GMP qualified process) and stored in a regulated environment, thawed, and then used when required. Alternatively, a small sample is separated from an existing stored unit when there is need for such a specific HLA-typed sample and this is then processed to generate an iPSC line for potential therapeutic use or when a cord blood unit is shipped for use a small sample is retained and iPSC lines are made to provide a replenishment for the used unit. Other methods of integration-free iPSC derivation also exist. These include protein-based methods, use of synthetic RNA, conditioned media, and excisable vectors (reviewed in ref. [41]). The efficiency of these methods using cord blood as a starting material remains to be determined.

The incentive for private cord blood banks is obvious. The procedure allows them to address the ethical issues of private storage by making available cells for the public good. It provides them with additional incentive to expand their storage efforts and extend the potential utility of their stored samples to treat a variety of additional conditions as pluripotent cells can theoretically be used to treat many more conditions than cord blood cells alone. Furthermore, pluripotent cells represent an insurance value if you will as technical advances suggest that one may be able to replace the UCB unit used for therapy with HSC derived from the pluripotent cells generated from that unit [34, 40, 41].

Likewise, the incentive for public banks is clear. The ability to make iPSC from UCB will allow them to recover some additional costs and enhance the utility of the samples collected and allow them access to the samples stored by private banks as well. Given that samples have been stored for many years, prospective history (from time of collection) available from donors may allow for an added layer or selection criteria that is unavailable with other samples used for iPSC generation.

## Summary

Clinical use of UCB has markedly increased over the past several years and this has led to the development of an entire banking industry. Scientific evidence has suggested that these cells are not only an alternative to BM but also perhaps even superior in some applications. The utility of UCB has been expanded by the findings that multiple units can be used, thus circumventing problems of small volume and HSC ($CD34^+$ cell) quantity. Studies to show that additional stem cell populations, in addition to hematopoietic stem/progenitor cells, may be present in UCB suggest that the future use of cord blood may be even greater in the future. For these reasons, it is important to advocate banking of UCB. As the field has evolved, there has been increasing controversy between public and private banking but we would argue that this controversy

overshadow the potential utility of this source of HSCs. Rather one should consider ways to make these stored units more widely accessible.

Developing additional uses of stored and freshly collected cord blood cells would represent an incremental cost for a potentially huge benefit. We propose that generating iPSC from cord blood has many advantages over conventional methods and that one can leverage the infrastructure of the cord blood banking industry to accelerate the transition to therapy. Wider collection of cord blood and targeted collection of marrow stem cells from individuals with appropriate genetic profiles will allow one to generate banks of iPSC customized for large-scale screening and therapeutic purposes. Equally important, providing private cord blood banks with an incentive to offer an opportunity to make a limited resource collected for the use of one individual more widely available will reduce the ethical controversy that has surrounded private cord blood banking. We hope this article stimulates conversation and perhaps leads to enterprising groups demonstrating the viability of this concept. Preemptive planning by existing private banks, for HLA typing, coordination of storage requirements between public and private banks, and working with the regulatory authorities to devise appropriate storage and banking processes to ensure regulatory compatibility would go a long way to resolve some of these practical issues related to implementing such a program.

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## Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest. Dr. Rao was a full-time employee of Life technology which supplies tools and reagents to stem cells scientists. Dr. Lars Ahrlund-Richter is a part-time employee of a biotechnology company focused on preclinical development of anti-cancer drugs. Dr. Kaufman participates in hematopoietic and cord blood transplant studies.

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