

Umbilical Cord Stem Cells

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Until recently, blood that remained in the umbilical cord and placenta after delivery was routinely discarded. Now that this blood is known to contain both hematopoietic stem cells and pluripotent mesenchymal cells, there has been a substantial increase in the clinical use and research investigation of umbilical cord blood in hematopoietic transplantation and regenerative medicine. Until now, standards for collection and processing were not well established. The debate continues regarding the private banking of autologous blood for "biologic insurance" versus public banking for access by the general population. Obstetricians should support the acquisition of cord units for public banking in their geographic location where cord blood banks have established collection procedures. Issues related to cost, quality control, and the need for ethnic diversity in public banks preclude the universal collection of units from all obstetric deliveries. Directed donation of cord blood should be considered when there is a specific diagnosis of a disease within a family known to be amenable to stem cell transplantation.

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The concept of using umbilical cord blood as a source of stem cells for hematopoietic transplantation was first proposed by Edward Boyse in 1983.¹ Subsequent experiments in irradiated mice revealed that murine blood from near-term and neonatal mice contained adequate numbers of hematopoietic progenitor cells to effect bone marrow recovery.² The first effort at establishing an umbilical cord blood bank was undertaken at Indiana University to harvest cells from the siblings of children needing transplants. Using one of these units, Gluckman et al³ performed the first related transplant with umbilical cord blood in a 6-year-old boy with Fanconi anemia in Paris, France. This was followed one year later by the first related umbilical cord blood transplant in the United States.⁴ Kurtzberg et al⁵ are credited with

performing the first successful unrelated umbilical cord blood transplant in the United States in 1994.

In 1991, the New York Blood Center established the first public bank for umbilical cord blood through funding provided by the National Heart, Lung, and Blood Institute of the National Institutes of Health (NIH).⁶ In 1996, the NIH awarded a multicenter grant to umbilical cord blood banks and transplant centers to study the safety and efficacy of umbilical cord blood transplants. Today, more than 6,000 hematopoietic transplants have been undertaken worldwide using umbilical cord blood for a growing list of indications (see the box, "Indications for Cord Blood Transplant"; Mark Walters, MD, Children's Hospital Oakland Research Institute and Joanne Kurtzberg, MD, Director Carolinas Cord Blood Bank at Duke, personal communication, June 5, 2005).

Almost 15 years after the first successful umbilical cord blood transplant, House Bill 2852 (H.R. 2852-Cord Blood Stem Cell Act of 2003) was passed in the United States Congress to appropriate 15 million dollars in fiscal year 2004 for the establishment of a national cord blood bank program. Although a similar bill was not passed by the Senate, the omnibus appropriations bill 2673 (H.R. 2673-Consolidated

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Indications for Cord Blood Transplant*

Thalassemias

- α -thalassemia intermedia (hemoglobin H disease)
- α -thalassemia major (hydrops fetalis)
- β -thalassemia major (Cooley's anemia)
- β -thalassemia intermedia
- E- β^0 thalassemia
- E-B+ thalassemia

Sickle Cell disorders

- Sickle cell anemia (hemoglobin SS)
- HbSC disease
- Sickle β^0 thalassemia
- Sickle B+ thalassemia

Oncologic Disorders

- Acute lymphoblastic leukemia
- Acute myeloid leukemia
- Chronic myeloid leukemia
- Autoimmune lymphoproliferative syndrome
- Burkitt lymphoma
- Cytopenia related to monosomy 7
- Familial histiocytosis
- Juvenile myelomonocytic leukemia
- Hemophagocytic lymphohistiocytosis
- Hodgkin's disease
- Non-Hodgkin's lymphoma
- Langerhans cell histiocytosis
- Lymphomatoid granulomatosis
- Myelodysplasia syndrome

Hematologic Disorders

- Amegakaryocytic thrombocytopenia
- Autoimmune neutropenia (severe)
- Congenital dyserythropoietic anemia
- Cyclic neutropenia
- Diamond Blackfan anemia
- Evan's syndrome
- Fanconi anemia
- Glanzmann's disease
- Hypoproliferative anemia
- Juvenile dermatomyositis
- Juvenile xanthogranulomas
- Kostmann's syndrome
- Pancytopenia

- Red cell aplasia
- Refractory anemia
- Schwachman Syndrome
- Severe aplastic anemia
- Systemic mastocytosis
- Severe neonatal thrombocytopenia
- Congenital sideroblastic anemia
- Thrombocytopenia with absent radius (TAR syndrome)

Immune Deficiencies

- Ataxia telangiectasia
- Cartilage-hair hypoplasia
- Chronic granulomatous disease
- DiGeorge syndrome
- Hypogammaglobulinemia
- IKK gamma deficiency
- Immune dysregulation polyendocrinopathy
- Mucopolidosis, Type II
- Myelokathesis
- X-linked immunodeficiency
- Severe combined immunodeficiency
- Adenosine desaminase deficiency
- Wiscott-Aldrich syndrome
- X-linked agammaglobulinemia
- X-linked lymphoproliferative syndrome

Metabolic Disorders

- Adrenoleukodystrophy
- Gaucher's disease (infantile)
- Metachromatic leukodystrophy
- Globoid cell leukodystrophy (Krabbe disease)
- Gunther disease
- Hermansky-Pudlak syndrome
- Hurler syndrome
- Hurler-Scheie syndrome
- Hunter Syndrome
- Sanfilippo syndrome
- Maroteau-Lamy Syndrome
- Mucopolidosis Types II, III
- Alpha mannosidosis
- Neimann Pick Syndrome, types A and B
- Sandoff Syndrome
- Tay Sachs Disease

* Personal communication: Mark Walters, MD, of Children's Hospital Oakland Research Institute and Joanne Kurtzberg, MD, Director Carolinas Cord Blood Bank at Duke



Appropriations) included 10 million dollars in funding in fiscal year 2004 to establish a national cord blood bank and charged the Department of Health and Human Services to contract with the Institute of Medicine (IOM) to make recommendations on the logistics of establishing such a national program. The IOM issued its final report on April 14, 2005.⁷ This article will review basic concepts of umbilical cord blood with emphasis on the recommendations of the IOM report.

PRIMER ON HUMAN LEUKOCYTE ANTIGEN TYPING

The human leukocyte antigens (HLA) were first discovered in 1958. There are 2 classes of antigens. Class I antigens are expressed on the surface of almost all nucleated cells in the human body. They have been subclassified as HLA-A, HLA-B, and HLA-C. Class II antigens are expressed on the surface of immune cells and can be induced in some other cell types. These have been subclassified as HLA-DR, HLA-DQ, and HLA-DP. Human leukocyte antigens play a major role in the immune recognition of foreign proteins by binding short peptides and presenting them to T lymphocytes.

The 6 major genes encoding the HLA antigens are located in the major histocompatibility complex on chromosome 6. Alleles of these genes vary in their nucleotide sequence, resulting in different protein transcription products and subsequently the expression of different HLA antigens. Because an individual receives one chromosome from each of his/her parents, there is a 1 in 4 chance of 2 siblings in a particular family sharing the same HLA type. If 2 siblings share one common chromosome (50% chance), they are said to be haploidentical.

The HLA genes are the most polymorphic of any found in the human genome, with hundreds of alleles being identified to date at each locus (Table 1).⁸ New alleles are being continuously discovered. Identified alleles for HLA-A, -B, and -DRB1 alone would allow

for over 45 billion combinations, 9 times the world's current population. There are 2 explanations for this. Many of these represent a null allele—a unique HLA antigen is not encoded by this nucleotide change. In addition, specific combinations of alleles occur together in a phenomenon known as linkage disequilibrium. This limits the number of HLA types that occur in the general population. However, HLA types vary considerably based on race and ethnicity.

Human leukocyte antigen typing can be performed through serologic methods using antisera from multiparous women (*low-resolution* typing). Newer DNA-based techniques have resulted in an abandoning of serology for typing. *Intermediate-resolution* typing will define a group of possible alleles that encode for a specific antigen; *high-resolution* typing uses DNA sequencing to determine the specific allele.

There is no accepted definition of a *match* for hematopoietic transplantation. In general, a “6-of-6” antigen match refers to compatibility at the HLA-A, -B, and -DRB1 loci (identical genes would be present on both chromosomes). Typically, matches at the HLA-A and -B loci are performed through intermediate DNA resolution techniques, whereas the HLA-DRB1 match is performed using high-resolution molecular methods. Although there are 12 HLA loci that could potentially impact a hematopoietic transplant, many transplant centers additionally match for the HLA-C and -DQB1 loci (potential “10-of-10” antigen match).⁹

The National Marrow Donor Program currently includes more than 5.5 million adult donors with an average of 27,000 new donors being added monthly.¹⁰ The chance of finding an unrelated adult donor match through the National Marrow Donor Program using low-resolution typing is estimated to be 88% for whites, 80% for Hispanics, and 78% for Asians. Matches for African Americans continue to be problematic, with only 59% being available by 2003. For a marrow or peripheral blood stem cell collection transplant using an adult donor, the median time from initiation of the formal search to donor selection was 51 days in 2003. Even then, an additional 4–6 weeks is required for final donor medical evaluation and scheduling of the transplant procedure. Although the median time-frames can be significantly shortened in urgent situations, given the aggressive nature of the hematopoietic disorders that are treated with transplant, many patients who initiate a formal search never actually proceed to transplant.

The National Marrow Donor Program is also affiliated with 15 umbilical cord blood banks and now contains an inventory in excess of 40,000 units.

Table 1. Known Human Leukocyte Antigen Alleles

Gene	Alleles (n)	Gene	Alleles (n)	Gene	Alleles (n)
A	396	DRB1	413	DQA1	28
B	699	DRB3	42	DQB1	66
C	198	DRB4	13	DPA1	23
DRA	3	DRB5	18	DPB1	119

Data from Institute of Medicine. Cord blood: establishing a national hematopoietic stem cell bank program. Available at: <http://www.iom.edu/report.asp?id=26386>. Retrieved September 28, 2005.



Nearly all patients will find a 4-of-6 match with the current inventory, while the majority will have a 5-of-6 match.¹⁰

PRIMER ON HEMATOPOIETIC STEM CELL TRANSPLANTATION

Since the first successful bone marrow transplant in 1968, hematopoietic progenitor cells have been used to treat a variety of leukemic disorders, hemoglobinopathies, and inborn errors of metabolism. Recipients must undergo ablative chemotherapy and sometimes total body irradiation before transplant to destroy disease and prevent the rejection of the donor cells. Factors that play a role in the success of the transplant include the ages of the patient and the donor, the disorder being treated, the premorbid condition of the patient, the degree of HLA mismatch, and the total number of stem cell and progenitor cells transplanted.¹¹ Initially, stem cell activity was measured by plating the hematopoietic progenitor cells product on methyl cellulose cultures to measure the colony-forming units after 10–16 days. Because of variability in the assays between laboratories, flow cytometry is now widely used to quantify hematopoietic progenitor cells that express the CD34 antigen on their surface (CD34+ cells). The assay to quantitate the number of CD34+ cells in umbilical cord blood has not been standardized. For this reason, cord units are primarily selected based on the measurement of the total nucleated cell count after processing. Engraftment after hematopoietic progenitor cell transplant is measured by the recovery of circulating neutrophils. Neutrophil recovery is usually defined as the time interval from the day of transplant to the first of 3 consecutive days with a circulating level of 500 neutrophils/mm³. Platelet recovery is usually defined as a count of 20,000/mm³ or more unsupported by transfusions for at least 7 days.¹² Donor T-lymphocytes in the hematopoietic progenitor cells product can attack the tissues of the recipient in a process known as graft-versus-host disease (GVH). Acute GVH occurs within the first 100 days posttransplant and is graded as I (mild) to IV (severe). Chronic GVH is graded as limited or extensive. Grades III and IV acute GVH occur in 18–50% of HLA-matched marrow recipients from unrelated donors; chronic GVH occurs in 55–75% of these patients.¹³ Residual T-lymphocytes in the hematopoietic progenitor cell product may also play a beneficial role in the patient with a hematopoietic malignancy; this is known as the graft-versus-leukemic effect.

ADVANTAGES AND DISADVANTAGES OF CORD HEMATOPOIETIC PROGENITOR CELLS

The use of stem cells from cord blood has several clear advantages over bone marrow donation or collection of peripheral stem cells from a donor (see box, “Advantages and Disadvantages of Cord Blood”). The establishment of a national cord blood stem cell program would allow easy access to many donors of a diverse racial and ethnic population. Units could be located on short notice through a computerized search. Because the unit is already tested and banked, the unit would be available in a short time interval. Recent data from the National Marrow Donor Program would indicate that the average time from initiation of a donor search to the request for the cord unit for transplant is less than 2 weeks (Christina Grier, National Marrow Donor Program, personal communication, September 8, 2005). In contrast, adult donors who can be found through a computerized registry at the National Marrow Donor Program may be difficult to locate because of a change in their geographic location or because they may decline to participate. In addition, the acquisition of bone marrow from an adult donor requires hospitalization and anesthesia and may be accompanied by postoperative pain (the bone marrow is usually aspirated from the pelvic crest under epidural anesthesia). Peripheral blood stem cell units are collected by outpatient apheresis procedures, but most donors

Advantages and Disadvantages of Cord Blood

Advantages

- Limitless supply
- Available on short notice for transplant
- No donor attrition compared with bone marrow registry
- Ethnic diversity easier to achieve
- Painless collection of stem cells
- Higher proliferative capacity
- Lower rate of acute graft-vs-host disease

Disadvantages

- Unable to obtain additional “donor” cells for leukocyte infusion or second transplant
- Fewer total HPCs due to small volumes
- Slower engraftment (return of circulating neutrophil and platelet numbers)
- Large inventory product (high up-front costs; units may become “outdated” due to changes in banking standards)



must first receive 4–5 injections of a mobilizing agent, most often filgrastim (Neupogen, Amgen Incorporated, Thousand Oaks, CA).

Studies of in vitro cultures of CD34+ cells from umbilical cord blood have yielded a higher rate of proliferation than similar cells from marrow.¹⁴ In addition these cells have a greater capacity for self-renewal and long-term growth in culture.¹⁵ Unfortunately, the greatest limitation to the use of cord blood appears to be the total cell dose (measured as either the total number of nucleated cells or the CD34+ count). This is predominantly related to the volume of blood that can be obtained from a placental collection. The result is that the transplanted cell dose is approximately 10% of a marrow transplant.¹⁶ The dose of cells needed to insure engraftment is subject to ongoing debate. The IOM report suggests that an “effective” unit is one with at least 2.5×10^7 nucleated cells per kilogram of recipient body weight.⁷ The lower cell dose in umbilical cord blood units was the determining factor for attempting transplants initially in children using this source of hematopoietic progenitor cells. As interest in using umbilical cord blood in adults has grown, experimental procedures, including ex vivo expansion of the cells and the use of multiple umbilical cord blood units in the same recipient, have been used. The issue of lower CD34+ cell numbers in umbilical cord blood units is thought to be responsible for the longer reported interval for both neutrophil recovery and platelet recovery (signs of engraftment) that occurs after umbilical cord blood transplants compared with marrow transplants. In one recently published study in adults, neutrophil recovery occurred a median of 7 days later in umbilical cord blood transplants compared with unrelated bone marrow transplants; platelet recovery was 60 compared with 29 days, respectively.¹² However, similar rates of treatment-related mortality, treatment failure, and overall mortality were reported. Adjusted 3-year survivals were 20% for unmatched bone marrow, compared with 26% for unmatched umbilical cord blood.¹²

A clear advantage of umbilical cord blood as a source of hematopoietic progenitor cell transplant is that a greater HLA mismatch is tolerated by the recipient than is the case with bone marrow transplant. Several biologic differences may explain this advantage. CD8+ lymphocytes, thought to be the predominant mediators of GVH, are reduced in numbers in umbilical cord blood.¹⁷ In addition, cord blood lymphocytes appear to express a more immature phenotype with a decreased ability to produce certain cytokines and an inability to generate cytotoxic effector cells.¹⁸ A recent report of outcome with hemato-

poietic transplantation using different donor sources included 367 recipients of HLA-matched bone marrow, 83 recipients of mismatched bone marrow, and 150 mismatched recipients of umbilical cord blood.¹² Acute GVH was similar between mismatched umbilical cord blood and matched bone marrow but was less likely to occur (relative risk 0.66) when umbilical cord blood was used. Compared with mismatched marrow, umbilical cord blood did not seem to demonstrate any advantage in the rate of chronic GVH.^{12,16} Because of this tolerance of HLA incompatibility, the current recommendations for a “matched” cord unit include 2 or fewer HLA disparities at the HLA-A, -B, and -DRB1 loci (a minimum of a “4-of-6” match).

The issue of greater tolerability of HLA-mismatch with umbilical cord blood transplants led to a theoretical concern for a reduced graft-versus-leukemic effect. However, subsequent studies have not substantiated this concern, with no detectable differences in rates of leukemia relapse when bone marrow transplants are compared with transplants using umbilical cord blood.^{12,16}

FUTURE USES OF CORD BLOOD

Perhaps the greatest future for cord blood lies in the possibility for its use for the regenerative treatment of disease. Although cord blood has proven to contain a high concentration of cells that can restore the hematopoietic system, the recent isolation of mesenchymal cells from cord blood has created new possibilities for tissue transplant.¹⁹ These cells have been described as *fetal stem cells* because they can be induced in culture to form a variety of tissues, including bone, cartilage, myocardial muscle, and neural tissue. More importantly, their acquisition from a readily available source does not involve the same controversies as embryonic stem cells from human conceptuses.

The majority of investigations to date using umbilical cord blood for regenerative therapy have been in experimental models for neurologic diseases. Neural and glial phenotype markers can be detected on donor cells that have engrafted in the brain in some studies.²⁰ Other studies have suggested that neurotropic factors found in cord blood may play a role in the improved function that is noted in the experimental models.²¹ In one animal study, human umbilical cord blood was injected into mice with iatrogenically induced intracranial hemorrhage. Control animals also underwent induction of intracranial hemorrhage but were treated with saline. By day 14, limb placement testing in umbilical cord blood-treated animals resembled controls.²¹ Intravenous injection of human



umbilical cord blood has also proven to delay the onset of neurologic symptoms and improve life expectancy in a mouse model for amyotrophic lateral sclerosis.²² Other potential neurologic diseases under investigation include spinal cord injury, Alzheimer's disease, Parkinson's disease, multiple sclerosis, and acute hypoxic brain injury. Inborn errors of metabolism that result in progressive neurologic deterioration, such as inherited leukodystrophies, Hunter syndrome, and Hurler syndrome have been treated successfully with umbilical cord blood transplant after myeloablative chemotherapy. Staba et al²³ treated 20 children with Hurler syndrome and noted that neurocognitive function stabilized or improved in all 15 of the 17 surviving children who were followed serially. McGraw et al²⁴ recently reported the results of umbilical cord blood transplantation in 15 newborns with Krabbe disease and demonstrated normal myelination and neurological development after transplant.

Other injured tissues may eventually be targeted with cells generated from umbilical cord blood. These include cardiac muscle (myocardial infarction), gastrointestinal epithelium (inflammatory bowel disease), and hepatocytes (toxic liver damage). In addition, umbilical cord blood may be used as a conduit for cell-based gene therapy.

ACQUISITION OF CORD BLOOD UNITS

The Consent Process

The report of the Institute of Medicine addressed several issues related to patient consent.⁷ It recommended that cord blood centers establish clear policies as to who must provide consent for donation. A plan to address paternal objection to the donation of cord blood should be developed. One of their additional recommendations was that balanced information for both autologous and allogeneic donation (donation to other individuals) should be provided to the pregnant patient in the antenatal period. In one study, almost one third of participants did not realize that they had the option to discard their cord blood at delivery while only 50% were aware that they could place their blood in a private bank.²⁵ Patients also should be informed that they relinquish property rights to a cord unit that is donated to a public bank. Sugarman et al²⁵ noted that half of respondents stated that the reason that they were donating to a public bank was to protect their child's future health. The IOM report also suggested that for public cord banking, the consent process should not include a promise

that the cells may be available at a later date for use by the family.⁷

An additional recommendation of the IOM was that the consent for the cord blood collection process should optimally be obtained before labor, preferably in the late third trimester. Although transplants using umbilical cord blood are clearly no longer investigative, at present public banks continue to use consents that are approved through local institutional review boards. Women may present in early labor without having previously completed the consent procedure but still wanting to make a cord blood donation. In these cases, a "miniconsent" can be signed in labor that allows a cord blood bank to collect a unit and obtain maternal blood samples for later testing. A full consent should then be obtained in the first 24 hours after delivery. This is especially important given the infectious disease screening (see below) that will be performed. The consent process should include disclosure for units that do not meet quality standards. Many of these units are used for quality control or may be sold or provided by cord blood banks for research purposes. The IOM also stated that patients should be assured that a secure link will be maintained between the unit and demographic data. This link is maintained for 2 reasons: new genetic testing may become available and a unit that is later being requested for transplant may test positive for a "new disease." In these situations, the IOM suggested that banks should make a reasonable effort to locate and notify the donor or parents.⁷ In addition, many banks contact patients before issuing a unit for transplant to assure the continued good health of the donor (the infant).

Cord Blood Collection

Several perinatal factors have been associated with increased nucleated cell counts in cord blood units. Higher numbers are associated with first-born infants, increased birth weight, prolonged labor, increasing gestational age, and white compared with African-American race.^{26,27} Smoking is associated with a decrease in counts, presumably through its association with lower birth weight.²⁸ A shortened interval to cord clamping and placing the infant on the maternal abdomen are associated with enhanced cell numbers.^{26,29} However, the IOM report strongly discourages any changes in routine obstetric practice to enhance the quality of a cord unit.⁷ In some reports the mode of delivery, vaginal versus cesarean, does not seem to influence the CD34+ count, whereas other studies indicate higher counts after cesarean delivery.^{30,31}



Cord blood is collected at the time of delivery by one of 2 techniques: either in vivo (while the placenta still remains in utero; Fig. 1) or in vitro in a specialized apparatus (Fig. 2). The cord is wiped clean and held slightly away from the perineum to avoid contamination with maternal blood. It is then prepped with povidone iodine and alcohol; a large bore needle is then inserted into the umbilical vein. This is connected to a closed collection bag that contains an anticoagulant (usually citrate-phosphate-dextrose). Obstetric providers new to cord collection should undergo standardized training on proper techniques. Ongoing quality assurance should be undertaken by the umbilical cord bank to ensure that the profiles of collectors do not point to consistent problems such as bacterial contamination or low volume units.

In vitro collection is usually undertaken by trained collectors outside of the delivery room at a specified location, usually in the Labor and Delivery suite. The distal end of the umbilical cord is clamped by the obstetric provider and the placenta delivered intact. Traction should be avoided to prevent tearing of umbilical vessels at the cord insertion because a break in these vessels results in the need to discard the unit due to the possibility of bacterial contamination. The placenta is then inserted into a holding device and the cord cleansed and punctured in much the same fashion as during in vivo collection.

A comparison of the 2 techniques has indicated larger unit volumes and higher total nucleated cell counts with in vivo collection.³⁰ This may be the result of placental collapse secondary to acute uterine involution after the delivery of the fetus. Alternatively, macroscopic clot formation may occur with the pro-

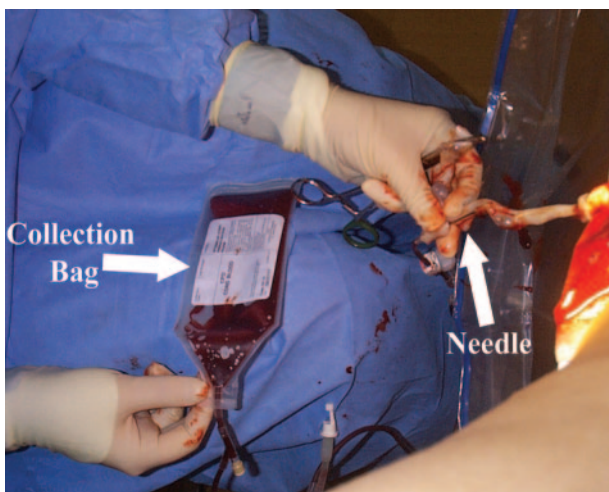


Fig. 1. In vivo collection of cord blood.
Moise. Umbilical Cord Blood. Obstet Gynecol 2005.

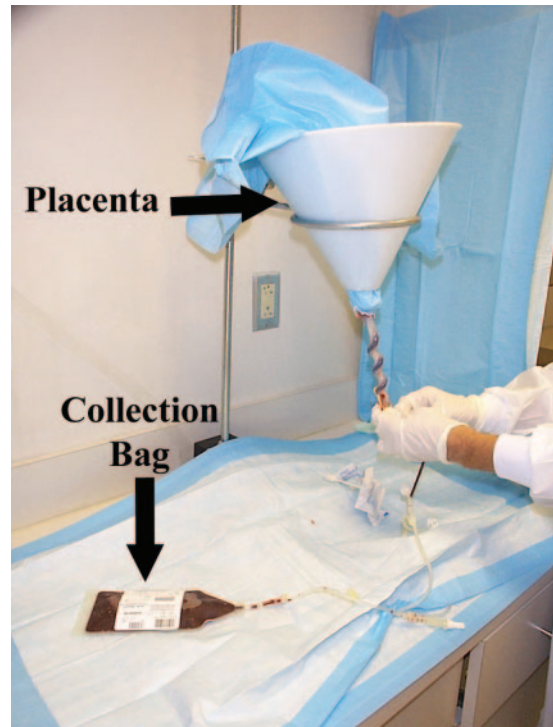


Fig. 2. In vitro collection of cord blood.
Moise. Umbilical Cord Blood. Obstet Gynecol 2005.

longed handling times necessary for in vitro collection.³² A higher incidence of units exhibiting bacterial contamination has also been reported with in vitro collection.³⁰ From a practical sense, in vivo collection adds minimal time to the delivery process after vaginal delivery. In vivo collection at cesarean delivery increases operative time and can make placental removal more difficult once the uterus has involuted.

Screening of Donors

In the case of donation for public use, donors must undergo extensive screening for both genetic disorders and infectious diseases. This usually includes a review of the obstetric events that might affect the quality of the cord blood unit. The following criteria, established by the National Marrow Donor Program, applies to events at the time of delivery (Christina Grier, National Marrow Donor Program, A centralized cord blood registry to facilitate allogeneic, unrelated donor umbilical cord blood transplantation. Version 4.1, personal communication, July 1, 2005):

- Cord blood is usually not collected from pregnancies of less than 34 weeks of gestation due to the lower total nucleated cell count counts associated with the smaller placental and infant size associated with earlier gestational ages.



- A positive carrier state for group B streptococcus, the presence of meconium, and prolonged rupture of membranes (in the absence of suspected maternal infection) are not considered exclusion criteria.
- Multiple gestations are usually excluded due to the possibility of cross contamination and issues with proper labeling of cord blood units at the time of delivery.
- Other exclusion criteria include suspected chorioamnionitis, a malodorous placenta, suspicion of or active genital herpes, extensive vaginal or perineal condylomata, or a tear of the placental plate vessels due to excessive traction. These factors may increase the likelihood for infection in the unit.
- Any chromosomal or major phenotypic structural abnormality of the neonate excludes a umbilical cord blood unit. All neonates should undergo a physical examination to detect more subtle anomalies that have been associated with congenital hematologic disorders.

Follow-up of the infant (the donor in umbilical cord blood collection) should be undertaken in the first few years of life. Some umbilical cord blood banks contact the parents by phone or mail a questionnaire at a prescribed interval after birth. In one survey of umbilical cord blood donors, only one fourth of respondents stated that they knew how to contact the bank if their infant became seriously ill.²⁵ More disturbing was that serious illnesses that occurred in mother-infant pairs were only reported to the bank in 2 of 7 cases in this study. Many banks routinely contact parents at the time a unit is being issued for transplant to assure that the donor child has not developed a disease such as leukemia or a metabolic storage disease that could be transmitted through cord stem cells.

At the time of umbilical cord blood donation, a thorough family history is reviewed for hematologic and immune abnormalities as well as various malignancies. Testing of the infant donor or the cord blood to exclude the presence of a homozygous hemoglobinopathy is required. Infectious disease screening is undertaken on the infant's mother. A thorough history is reviewed to exclude overseas travel to specific countries, exposure to live viral vaccines, use of illicit drugs, or high-risk sexual behavior. Infectious serologies for viral and bacterial disease, as required by the U.S. Food and Drug Administration (FDA) for any cord blood donation, are drawn at the time of admission to the Labor and Delivery suite or in the immediate postpartum period. These include testing for

hepatitis B and C, human immunodeficiency virus (HIV) 1 and 2, human T-lymphotropic virus (HTLV) 1 and 2, West Nile virus, and syphilis.

PROCESSING OF BLOOD

Cord blood can be stored at room temperature for up to 48 hours with minimal effect on cell viability.³³ Samples from the unit are sent for bacterial culture, red blood cell type, preliminary HLA testing, and cell counts. The unit is processed by first adding a Hespan solution to facilitate separation of the red cells from the mononuclear white cells at the time of centrifugation. The plasma is extracted to result in a final volume of approximately 20 mL. Generally the unit is mixed with a final concentration of 10% dimethyl sulfoxide, cryopreserved by controlled rate freezing, and then stored in liquid nitrogen at -196°C . Approximately 20% of the hematopoietic progenitor cells are lost through the thawing process.¹¹ Multiple segments are attached to the specialized storage bag to allow for confirmatory HLA testing without subjecting the unit to prolonged periods of thawing in case the unit might later be requested by a transplant center.

REGULATIONS

The cord blood banking industry has been surprisingly unregulated since its inception. In January of 2005, Bone Marrow Donors Worldwide reported the U.S. inventory of public cord blood banks from unrelated donors to be in excess of 87,000 units. However, almost half of these units probably do not meet criteria for a usable unit based on cell count and other collection issues.⁷ McCullough et al³⁴ reviewed the quality of 268 umbilical cord blood units received at their transplant center between the years 1994 and 2004 from cord blood banks in the United States and Europe. Fifty-four percent of units were determined to have quality control issues, including 21 units with incomplete or positive testing for transmissible infectious diseases and 4 units with bacterial contamination. Ten percent of the quality control issues were felt likely to affect the overall quality of the unit. An additional 40% of units had problems with documentation of medical history (4% thought to affect unit quality), and 6% of units had problems with labeling and documentation (4% thought to affect unit quality).

Although active in the regulation of donated adult blood products in the United States, the FDA has only recently become involved in the regulation of cord blood banking. The reasons for this are unclear although private banks have lobbied extensively against regulation of the industry. Many banks have undergone voluntary accreditation through the American Association of Blood Banks or the NetCord/



Foundation for the Accreditation of Cellular Therapy (FACT). Both organizations have developed specific guidelines for cord blood banking, and both conduct site inspections of the collection facilities. The National Marrow Donor Program has also established standards for cord blood bank participation in its network, providing annual assessments and biannual audits of member banks. In 1997, the FDA proposed regulations for cellular and tissue-based products that included the cord blood banking industry. In January 2004, all facilities collecting cells for hematopoietic transplant were required to register with the FDA. This regulation included private banks. Finally, on May 25, 2005, FDA regulations (21 CFR 1271) for cord banking were passed. Although routine inspections are not planned at this time because of a lack of resources, under this new regulation, cord blood banks must notify the FDA of specific adverse reactions in the stem cells they process and allow for FDA inspections. Private banks that involve the collection of autologous cord blood units or cord blood units to be used by a primary family member are currently exempt from the new FDA regulations. The IOM report contained several definitive proposals regarding quality assurance and accreditation of cord blood

banks that would participate in a national cord blood program.⁷ Specifically, the Health Resources and Services Administration should identify and contract with one of the existing organizations that accredit banks to establish uniform standards for collection and quality assurance. Such standards would apply to both public and private banks that participate in the program. It also recommended that the FDA establish a system of licensure of cord blood units intended for clinical transplantation.

TYPES OF CORD BLOOD BANKS

There are 3 types of cord blood banks: public banks, private banks, and directed-donation banks. Public banks involve allogeneic donation. At the time this article was written, there were a total of 22 public cord blood banks operating in the United States (Tables 2 and 3).^{35,36} In these situations, blood is collected from the general public in a manner analogous to whole blood donation. The stem cells are then stored in a central facility for public use. These units must meet rigorous standards for infectious disease testing identical to the blood donor pool for adult blood. Initial HLA testing, red cell blood type, and cell counts are performed. Units that do not meet certain criteria for

Table 2. U.S. Public Cord Blood Banks (National Bone Marrow Donor Program)*

Bank	Location	Web Site
American Red Cross Western Area Community Cord Blood Bank	Portland, Oregon	http://chapters.redcross.org/ca/norcal/donating/beacord.htm
Ashley Ross Cord Blood Bank Program of San Diego Blood Bank	San Diego, California	http://www.sandiegobloodbank.org
Bonfils Cord Blood Services Belle Bonfils Memorial Blood Center	Denver, Colorado	http://www.bonfils.org
COBLT Cord Blood Units Carolinas Cord Blood Bank	Durham, North Carolina	http://cancer.duke.edu/CCBB/
Carolinas Cord Blood Bank Children's Hospital of Orange County Cord Blood Bank	Durham, North Carolina Orange, California	http://cancer.duke.edu/CCBB/ http://www.choc.org
ItxM Cord Blood Services	Glenview, Illinois	http://www.itxm.org/stemcell.htm
J. P. McCarthy Cord Stem Cell Bank	Detroit, Michigan	http://www.karmanos.org
LifeCord	Gainesville, Florida	http://www.lifesouth.org/lifecord/lifecord.htm
New Jersey Cord Blood Bank at the Coriell Institute of Medical Research	Camden, New Jersey	http://www.coriell.org/njcb
Puget Sound Blood Center	Seattle, Washington	http://www.psbcc.org/cordblood/
St. Louis Cord Blood Bank	St. Louis, Missouri	http://www.slcbcc.org
StemCyte International Cord Blood Center	Arcadia, California	http://www.stemcyte.com/

* All National Marrow Donor Program banks collect at specific hospitals in their state/region. Data from National Marrow Donor Program. Cord blood banks in the NMDP network. Available at: http://www.marrow.org/cgi-bin/NETWORK/nmdp_cord_blood_banks.pl. Retrieved September 29, 2005.



Table 3. Other U.S. Public Cord Blood Banks

Bank	Location	Web Site	Collection Strategy
Children's Hospital Oakland Research Institute	Oakland, California	http://chori.org/siblingcordblood	Collects from any hospital in the U.S. when there is a child with a condition treatable by transplant
University of Colorado Cord Blood Bank	Aurora, Colorado	www.coloradocord.org	Collects only at Poudre Valley Hospital in Fort Collins
Cryobanks International	Altamonte Springs, Florida	www.cryo-intl.com/	Accepts donations from anywhere in the continental United States
Michigan Community Blood Centers Cord Blood Bank	Grand Rapids, Michigan	http://www.miblood.org/giving_blood/cordblood.html	Collections at only 12 specific hospitals in the state of Michigan
LifeBank USA	Cedar Knolls, New Jersey	www.lifebankusa.com	Collections accepted from any hospital in New Jersey
The Ellie Katz Umbilical Cord Blood Program	Paramus, New Jersey	www.communitybloodservices.com/cord_blood_1_program.htm	Collections accepted from most New Jersey hospitals
The New York Blood Center/National Cord Blood Program	New York, New York	http://live.nybloodcenter.org/about/products/index.do?sid0=5&sid1=39	Collection sites at 3 hospitals in New York and one in Virginia
Ireland Cancer Center at Case Western Reserve University and University Hospitals of Cleveland Umbilical Cord Blood Program	Cleveland, Ohio	http://www.irelandcancercenter.org	Collections accepted only from University Hospitals of Cleveland
South Texas Blood and Tissue Center	San Antonio, Texas	http://www.bloodntissue.org/texascordbloodbank.asp	Collections accepted only from San Antonio hospitals

Data from National Marrow Donor Program. Non-NMDP cord blood banks. Available at: http://www.marrow.org/NMDP/non_nmdp_cord_blood_banks.html. Retrieved September 29, 2005.

cell count or volume are not included in the active inventory. Funding for the establishment of a public cord blood bank is problematic. Initial processing costs typically exceed \$1,000 per unit stored. Most current public banks were initiated with research funding from the NIH or funding from local foundations. Recently the National Marrow Donor Program has subsidized cord blood banks in its member network. Public banks are allowed to recover some of their costs by charging insurance carriers for units used for transplant. Fees usually are on the order of \$15,000 to \$35,000 per unit (average \$25,000). Sirchia et al³⁷ studied an economic model for the initiation of a public cord blood bank. The establishment of an inventory of 10,000 units was proposed during the first 3 years. In years 4–7, only 3% of the inventory would be released for transplant, necessitating a

charge per unit of approximately \$12,000 to make the venture cost neutral.

Private banks were initially conceived for autologous use by a child that develops a disease later in its life. More recently private banks have promoted their use for allogeneic donation for siblings or parents. Some private banks offer directed donation at no charge to the patient if there is a sibling or parent with a known disease that can be treated with umbilical cord blood.³⁸ Today there are more than 24 private banks established in the United States (Table 4).³⁹ In general, units for private banks are collected on site by an obstetric provider and shipped to a central processing laboratory. Because these units are being collected primarily for autologous use, most banks limit their testing for maternal infectious diseases. Initial HLA typing is not undertaken. Families are



Table 4. Private Cord Blood Banks

Bank	Location	Web Site
Alpha Cord, Inc.	Atlanta, Georgia	www.alphacord.com
CellMed Biotech	Paramus, New Jersey	www.cellmedbiotech.com
CorCell	Philadelphia, Pennsylvania	www.corcell.com
Cord Blood Family Trust	Arcadia, California	www.cordbloodfamilytrust.com
Cord Blood Registry (CBR)	San Bruno, California	www.cordblood.com
Cord Blood Solutions	Alpharetta, Georgia	www.cordbloodsolutions.com
Cord Partners, a Cord Blood America Company	Los Angeles, California	www.cordpartners.com
Cryobank for Oncologic and Reproductive Donors, Inc.	Middletown, New York	www.nycryobank.com
Cryobanks International	Altamonte Springs, Florida	www.cryo-intl.com
Cryo-Cell International	Olsmar, Florida	www.cryo-cell.com
CureSource	Charleston, South Carolina	www.curesource.net
Family Cord Blood Services	Santa Monica, California	www.familycordbloodservices.com
Family Link Cord Blood Storage Program	Louisville, Kentucky	www.nortonhealthcare.com/specialities/women/obstetrics/cord_blood_storage.aspx
Genesis Bank	Indianapolis, Indiana	www.thegenesisbank.com
HemaStem Therapeutics	Hamilton, ON, Canada	www.hemastem.com
LifeBankUSA	Cedar Knolls, New Jersey	www.lifebankusa.com
LifeLine Cryogenics	Stamford, Connecticut	www.lifelinecryogenics.com
MAZE Laboratories	Purchase, New York	www.MAZElabs.com/cordblood.htm
National Children's Leukemia Foundation	New York, New York	www.leukemiafoundation.org/stemc/stemc.htm
Newborn Blood Banking, Inc.	Tampa, Florida	www.newbornblood.com
New England Cord Blood Bank, inc.	Boston, Massachusetts	www.cordbloodbank.com
Securacell, Inc.	Canton, Ohio	www.securacell.com
Stembanc	Cleveland, Ohio	www.stembanc.com
Viacord	Boston, Massachusetts	www.viacord.com

Data from Parent's Guide to Cord Blood Banks. Private cord blood banks in the USA. Available at: <http://www.parentsguidecordblood.com/content/usa/banklists/listusa.shtml?navid=10>. Retrieved November 1, 2005.

charged an initial fee (\$1,100–\$1,750) followed by a yearly fee for continued storage (\$115–\$125).⁴⁰ If a cord blood unit should later be needed, processing and shipment fees are billed to the health care insurance carrier.

A directed-donation public bank is one in which cord blood is collected at no charge to the patient in situations where a sibling is affected with a disorder in which cord blood transplant may prove beneficial (see the box, "Indications for Cord Blood Transplant"). The Children's Hospital Oakland Research Institute is currently the only federally funded bank of this type.

THE PUBLIC VERSUS PRIVATE BANK CONTROVERSY

The banking of umbilical cord blood for private use or public use is mired in emotion with very few facts. Private companies, particularly in the United States, have used direct patient advertising for recruitment, often using a promise of "biologic insurance" for the newborn. One company even offers a college savings plan as part of their package for storing cord stem

cells.⁴¹ Important issues of future use, quality control, long-term availability, availability to those in need, and costs argue for public banking as a more practical approach to the use of umbilical cord blood.

In its committee opinion on this issue, the American College of Obstetricians and Gynecologists (ACOG) states "Parents should not be sold this service without a realistic assessment of their likelihood of return on their investment."⁴² Some private banks quote unrealistic odds for the future use of an umbilical cord blood unit stored in a private bank. One private bank cites a frequency of 1:27, with the future possibility of 50% of units ultimately being used.⁴³ Autologous umbilical cord blood cannot be used to treat inborn errors of metabolism because the genetic mutation is already present in the stem cells. In addition, some subtypes of leukemia are associated with chromosomal translocations that have been found in fetal blood.⁴⁴ For this reason, many pediatric hematologists will not use autologous stem cells to treat leukemia. In addition, the use of such cells would negate the beneficial graft-versus-leukemic effect (see above) that occurs with allogeneic stem cell trans-



plants. This led Johnson⁴⁵ to suggest that the chance of an individual using an autologous unit of cord blood is approximately 1:2,700 (Fig. 3).

The definition of a “quality” umbilical cord blood unit is still being refined. Initial procedures developed in the NIH COBLT trial called for a minimum unit volume at the time of the collection of 60 mL or a total nucleated cell count of 6×10^8 or greater if the volume was between 40 and 60 mL; units with volumes of less than 40 mL were discarded.⁴⁶ Public banks also have numerous other exclusion criteria that are meant to assure quality. Many banks now use a minimum of 1×10^9 total nucleated cell count to define an adequate unit; this results in as many as 65–70% of units being discarded after initial collection (Joanne Kurtzberg, MD, Director, Carolinas Cord Blood Bank at Duke, personal communication, July 1, 2005). The collection of units for private banking is subject to the pressure of “our only chance to collect cells.” Therefore, suboptimal units are often sent to the collection facility. At the time of the processing, the private bank will usually contact the parents to have them decide whether to store or discard the unit. Many decide to proceed with storage with very little knowledge that the unit could not be realistically used at a later date.

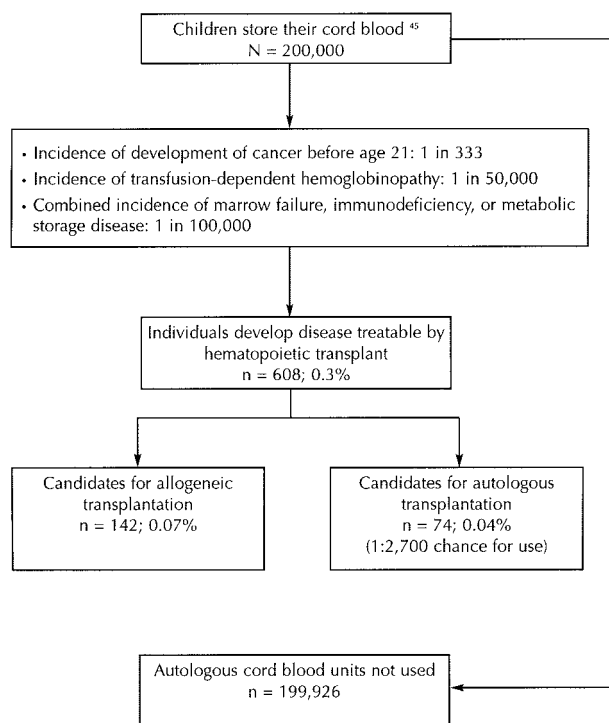


Fig. 3. Flow diagram for potential future autologous use of stored umbilical cord blood.

Moise. *Umbilical Cord Blood*. *Obstet Gynecol* 2005.

Because umbilical cord blood banking is in its infancy, issues with long-term availability have not arisen. Studies have shown long-term survival of stem cells in cord units for up to 15 years after initial freezing.⁴⁷ Viability of cells after this time has not been substantiated. This, therefore, calls into question the use of autologous stem cells harvested at birth for regenerative medicine many decades later. In addition, private banks must continue to recruit new donors to remain financially viable. What is to happen to privately donated units if a company becomes insolvent?

The final argument for public banking involves the use of a human resource for the greater good of mankind. Public banks collect units from patients with a wide ethnic diversity. Many actually seek out certain ethnic groups that are underrepresented in the national bone marrow registry. Private banking allows those of means to collect stem cells while the less fortunate have no access to this valuable resource. Because of the economics of maintaining a public bank based on current use, many public banks have found it necessary to curtail or even eliminate collection activity (this includes an initial effort by the Red Cross to establish a cord blood bank). The IOM report recommended federal funding for the acquisition of 100,000 new high quality units.⁷ Recently, the Senate Health, Education, Labor and Pensions Committee approved a bill (S.1317) to establish a national cord blood bank.⁴⁸ The bill would authorize the use of 19 million dollars already set aside by Congress to support inventory growth in the years 2005 and 2006 and directs an additional 15 million dollars to be set aside each year between 2007 and 2010 to establish the national inventory suggested by the IOM report. The bill would also consolidate the current national bone marrow registry and the new cord blood registry under a newly created C. W. Bill Young Cell Transplantation Program. The bill is expected to pass the House without going to conference committee and will be sent to President Bush for signature.

All of these reasons have led many organizations and countries to take a stand against private banking. The American Academy of Pediatrics has suggested “. . .private storage of cord blood for *biologic insurance* is unwise.”⁴⁹ In Europe, the practice of private cord banking has been banned by law in Italy since 2002. The Royal College of Obstetricians and Gynaecologists states their position as “Routine directed commercial cord blood collection and stem cell storage cannot be recommended at the present time, because of the insufficient scientific base to support such practices. . .”⁵⁰ The French National Consultative Ethics Committee’s “. . .recommendation to decision



makers is that they should encourage a considerable extension of cord public banks for essentially allogeneic purposes, rather than subscribing to the creation of private banks for strictly autologous purposes, the potential therapeutic usefulness of which is, as yet, in no way corroborated.⁵¹ Finally, in March of 2004, the European Group on Ethics in Science and New Technologies stated their position as follows: "The legitimacy of commercial cord blood banks for autologous use should be questioned as they sell a service which has presently no real use regarding therapeutic options. Thus, they promise more than they can deliver. The activities of such banks raise serious ethical criticisms."⁵²

LEGAL CONCERNS

Before the realization of the value of cord blood, obstetric providers routinely allowed the placenta to drain into the kick bucket at the foot of the delivery bed. The discovery of the value of this resource has created the possibility of new legal dilemmas, many of which have gone unrealized to date.

Parents of the newborn have limited rights to control their child's umbilical cord blood. However, the newborn has not legally abandoned its property rights to his/her own cord blood.⁵³ Because cord banking is still in its infancy, no newborn has reached the age to legally request ownership of his/her stem cells that may be stored in a public bank. The possibility that such a situation may occur in the future is not unrealistic.

Most private banks indemnify the collecting obstetric provider from any errors that may occur in the collection procedure at the time of delivery. One may conjecture a situation where a poor quality unit (low cell count or bacterial contamination) results from a collection. In a future life-threatening situation for the family, would the obstetric provider be held partially liable for suboptimal collection?

Finally, patent law has been the subject of considerable debate in the cord blood industry. An initial patent (U.S. patent 5,004,681) was filed in November 1987 by the Biocyte Corporation regarding the cryopreservation of neonatal and fetal blood and their therapeutic use for hematopoietic reconstitution after thawing. Subsequent patents were filed in November 1988 (U.S. patent 5,192,553), May 1990 (U.S. patent 6,461,645), and May 1995 (U.S. patent 6,569,427). The latter 2 patents were filed under the new entity of PharmaStem Therapeutics Inc, which also acquired the rights to the first 2 patents. International patents were issued to the company in Europe and Japan, but challenges were raised that the company had done nothing

more than demonstrate that the cells could be isolated and deep frozen. Subsequently, these initial patents were overturned. In the United States, PharmaStem licensed 14 private banks under agreements for undisclosed royalties at the time each unit was collected.⁵⁴ The company filed a lawsuit in federal court in Delaware against the remaining 5 private companies that did not agree to royalty payments. In October 2003, a jury upheld the argument that PharmaStem's patents were enforceable and willingly infringed by 4 of the 5 private cord blood banks, one having settled with PharmaStem before the jury award. The judge initially set aside the verdict. In June 2004, the company sent a warning letter to obstetricians indicating that they were infringing on the patents if they collected cord blood for 1 of the 4 remaining companies: Viacord, Cord Blood Registry, CRYO-CELL International, and Corcell. Three months later, the Federal District court of Delaware affirmed the jury verdict as to patentability but threw out claims for damages against banks for other reasons. All issues raised at trial are currently under appeal.

In separate actions to seek review of the patents at the United States Patent and Trademark Office, the Patent Office in 2005 revoked the PharmaStem patent describing collection, processing, and storage, but it upheld the patent describing the therapeutic use of cord blood. These rulings are under appeal as well.

Although private banks have been the target of these patent infringement cases, the first public bank to obtain a license from PharmaStem was the newly formed umbilical cord blood at M. D. Anderson Cancer Center in April 2005. Unfortunately, this latest event may open the door to additional costs for the public banks as this new technology comes into widespread use. Because public banks do not collect fees at the time of collection of the units, it is likely that royalties will be paid at the time units are shipped or thawed for transplant.

CONCLUSIONS

Umbilical cord blood represents an exciting new source of hematopoietic stem cells. The obstetrician represents the first line of information and counseling for the pregnant woman regarding the pros and cons of public versus private banking. Several key points should be considered:

- Pregnant women should be provided balanced information about both private and public cord blood banking during their prenatal course.
- The currently estimated chance of a child requiring a transplant with its own cord blood is 1:2,700. Promising research in regenerative medicine may allow for more applications of



autologous cord blood in the future. Many of these applications are, to date, unproven in clinical trials. The long-term viability of stored cord stem cells over many decades for such purposes is untested.

- Private banks for autologous storage are under less stringent FDA regulations for quality control than public banks.
- Legal issues related to patent infringement continue to cloud the collection of cord blood units for private banks.
- Public banks allow for greater access to cord blood by the general population, are more cost-effective, and allow for the establishment of ethnic diversity of their inventory. In situations where a hospital is not a collection site for a public bank, several banks will accept patient donations that can be shipped to their storage facility (Table 3).
- Directed donation of cord blood should be considered when there is a specific diagnosis of a disease within a family that is known to be amenable to stem cell transplantation. This can be arranged through many public banks, such as the Children's Hospital Oakland Research Institute.

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