Improving Quality and Potency Testing for Umbilical Cord Blood: A New Perspective

IVAN N. RICH
HemoGenix, Inc., Colorado Springs, Colorado, USA

SUMMARY
This article critically reviews current methods to test and characterize umbilical cord blood (UCB) for hematopoietic stem cell transplantation. These tests include total nucleated cell (TNC) count, viability, viable CD34-positive content, and the colony-forming unit assay. It is assumed that the data obtained are sufficient to perform a UCB stem cell transplant without actually determining the quality and potency of the stem cells responsible for engraftment. This assumption has led not only to a high graft failure rate attributed to low or lack of potency, but also to noncompliance with present statutes that require UCB stem cells to be of high quality and, indeed, potency for a transplant to be successful. New evidence now calls into question the quality of the data, based on the UCB processed TNC fraction because using this impure fraction masks and significantly underestimates the functionality of the stem cells in both the segment and the unit. It is proposed that UCB units should be processed to the mononuclear cell fraction and that new cost-effective technology that measures the quality and potency of UCB stem cells be implemented to achieve better practices in UCB testing. These changes would provide the transplant physician with the assurance that the stem cells will perform as intended and would reduce risk and increase safety and efficacy for the patient. Stem Cells Translational Medicine 2015;4:967–973

SIGNIFICANCE
Current stem cell transplantation of umbilical cord blood cells requires testing that includes four basic parameters that do not determine whether the stem cells are of high quality, as required by the Stem Cell Therapeutic and Research Act of 2005. No cord blood units collected or transplanted so far have been tested for stem cell quality or potency. New scientific evidence calls into question cord blood processing and testing practices required by regulatory agencies and standards organizations. A new perspective is described that includes stem cell quality and potency testing that could reduce graft failure rates.

INTRODUCTION
This paper provides a critical appraisal of the methods currently used to test and characterize umbilical cord blood (UCB) for stem cell transplantation. It would be remiss, however, not to counteract this appraisal with new scientific perspectives that might help improve the criteria to store UCB units and measure the quality and potency of the stem cells, on which the whole transplantation procedure is dependent.

Beginning in the 1970s, bone marrow hematopoietic stem cell transplantation became a routine procedure [1, 2]. This was followed by mobilized peripheral [3] and umbilical cord blood [4, 5] as alternative sources of stem cells. In the late 1990s, the U.S. Food and Drug Administration (FDA) initiated an approach to regulate human cellular and tissue-based products. In 1998, the FDA requested information to establish “Standards for Unrelated Allogeneic Peripheral and Placental/Umbilical Cord Blood Hematopoietic Stem/Progenitor Cell Products” [6] and considered a number of measures of quality. In 2005, the law “To provide for the collection and maintenance of human cord blood stem cells for the treatment of patients and research, and to amend the Public Health Service Act to authorize the C.W. Bill Young Cell Transplantation Program,” also known as the Stem Cell Therapeutic and Research Act of 2005, was enacted in the U.S. [7]. This statute provided funding that included, among other things, the establishment of a National Cord Blood Inventory (NCBI) by the National Marrow Donor Program (NMDP), an Advisory Council for Blood Stem Cell Transplantation (ACBSCT), and funded public cord blood banks (CBBs) to collect and maintain “high-quality umbilical cord blood units.” The term “high quality” refers to the title of this statute, in which the stem cells in the UCB unit have to be of high quality. The culmination of efforts to regulate the use of UCB for allogeneic transplantation occurred in 2009. Unrelated allogeneic hematopoietic stem/progenitor cells were considered to have a systemic effect, and UCB was designated as a drug by the FDA [8]. This has required CBBS to file a Biologics License Application, the guidelines for which were updated in 2014 [9]. There are approximately 30 public CBBS in the U.S., of which 5 have been approved by the FDA to date.

Harmonization efforts led to the FDA defining “non-binding recommendations” for UCB testing [8, 9]. Standards organizations worldwide, including AABB and NetCord-FACT, followed...
suit, recommending four basic tests to characterize the cells of a UCB unit, including total nucleated cell (TNC) count, viability, viable CD34 content, and the presence of granulocyte-macrophage (GM) progenitor cells detected using the colony-forming unit (CFU) assay [10, 11]. These parameters are referred to in this paper as “minimum testing criteria.” NetCord-FACT standards state that “These Standards are designed to provide minimum guidelines for Cord Blood Banks,” and declares that “These Standards are not intended to establish best practices” [11]. As a result, CBBs have had little incentive to implement better practices by measuring the stem cells required for engraftment because this would require greater effort to qualify for accreditation and licensure. The consequence has been noncompliance with the Stem Cell Therapeutic and Research Act that requires UCB stem cells to be of high quality [7].

**CURRENT PRACTICES AND PERSPECTIVES**

**Two Assumptions on Which Umbilical Cord Blood Stem Cell Transplantation Is Based**

In order to understand how UCB testing could be improved, it is necessary to consider current practices. Figure 1 shows a summary of the procedures and events leading to a cord blood transplant. The left side of the diagram illustrates present tests and assays and when they are performed. As of 2014, public CBBs worldwide have collected more than 730,000 UCB units, and more than 35,000 allogeneic cord blood transplants have been performed [12, 13]. Of these, more than 200,000 units have been collected and 5,000 allogeneic transplants performed in the U.S [14]. Cell count and/or volume are the primary criteria for storing a UCB unit by a public CBB [14]. Based on these criteria, only 10%–25% of all UCB units collected are actually stored [12, 15]; however, considering that all units are tested based on minimum testing criteria, virtually none of the units have been tested to ensure high quality and potency of the stem cells. This is because it is assumed that functional stem cells are present in the unit without actually measuring them. Once a unit is eligible for storage, a second assumption is made, namely, that tests performed after processing but before freezing will define the quality and potency of the post-thaw product. The prefreeze UCB unit parameters are uploaded to the cord blood inventory, and it is usually this information that is used by the transplantation centers (TCs) to help identify a UCB unit for transplantation. Some CBBs and TCs may perform minimum testing of the cells from a segment prior to shipment or use in a patient, respectively [16, 17]. To help predict potency of the UCB unit, a Cord Blood Apgar has been suggested that provides an arbitrary score by combining the prefreeze and post-thaw results [17, 18]. The Apgar might be useful in predicting between UCB units with high or low probability of a successful initial clinical outcome or time to neutrophil engraftment, but, as discussed below, it cannot predict potency because neither the stem cells nor the properties that define potency have been determined (Fig. 1).

**Minimum Testing Criteria and Their Limitations**

The FDA designates TNC count, viability, and viable CD34 content as “purity and potency” tests [8, 9]. Cord blood processing usually involves a plasma and/or red blood cell (RBC) reduction prior to cryopreservation [19]. Many cells die after thawing, but the TNC fraction is still impure, containing varying concentrations of RBCs, nucleated RBCs, granulocytes, platelets, and other cells. The CD34-positive (CD34+) “stem cell marker” only detects a very small proportion of stem cells [17, 20, 21]; the majority of cells detected by CD34 are hematopoietic progenitor cells that play no role in the engraftment process [17]. Even aldehyde dehydrogenase, which has been considered a marker for stem cells [22], also correlates with CD34+ and GM-CFU progenitor cells [23, 24]. Consequently, it is difficult to understand how the TNC count, viability, and CD34 content can be considered measures of purity let alone potency. Nonetheless, the decision to permanently store a UCB unit for transplantation purposes and enter the information into a cord blood registry or inventory is not based on the quality or potency of the stem cells but primarily on the TNC count, which must be at least 1 × 10^8 cells [25]. In fact, UCB units containing a TNC greater than 1.8 × 10^8 are now preferred by TCs [25, 26]. The reason is simple: transplant physicians want to use the largest UCB units because the higher TNC count, the greater the probability of a positive initial clinical outcome. It is assumed that a higher number of stem cells will be transplanted without actually measuring their quality or potency; however, this assumption is not always correct because the UCB graft failure rate attributed to low or lack of potency [18, 27] is between 20% and 24% [27–29], which even the Cord Blood Apgar has not been able to reduce [18].

Because all regulatory agencies, including the FDA, agree that potency is a quantitative measure of biological activity [30, 31], it is uncertain why TNC, viability, and CD34, which do not measure biological activity, are designated tests of potency [8, 9]. This approach is contradictory to the FDA’s own potency assay regulations [30]. Only the CFU functional assay can provide any relevant biological information. Performing this assay is a requirement by standard organizations [10, 11, 32]; however, this is also problematic for several reasons. First, high coefficients of variation (CVs) [32–35] and lack of standardization and validation [35, 36] produce low precision and robustness among laboratories and thus low credibility for the assay. Second, depending on the methylcellulose formulation, it is often assumed that the stem cell population colony-forming cell-granulocyte, erythroid, macrophage, megakaryocyte (CFC-GEMM) is being assessed when, in fact, the growth factor cocktail does not contain thrombopoietin to induce megakaryopoiesis [25]. Consequently, rather than a primitive hematopoietic stem cell population (CFC-GEMM), a more mature stem cell population is detected. Only two of three hematopoietic lineages are detected, and they provide only limited information related to short-term engraftment. Third, many laboratories only count and report GM colonies because this information is used to correlate with time to neutrophil engraftment. In addition, the NMDP inventory requirements for new UCB units stipulate a CFU postprocessing assessment and results documented as “growth” or “no growth” with an actual count, which can include all colonies or just GM colonies [32]. These and other minimum testing criteria are assumed to be potency assays [10, 11]. By definition, a potency assay must be validated to produce trustworthy results [30]. None of the minimum testing criteria, including the CFU assay, quantitatively measures the biological activity of the “active” stem cell components and conforms to other potency assay requirements [30]. As a result, there is little trustworthy scientific information on which a CBB can release a unit and a transplant physician can make an informed decision about whether to transplant the unit, other than compatibility and the assumption that sufficient stem cells are present in a high TNC count unit. Consequently, most of the data, including CFU results, are used retrospectively to correlate with clinical
Figure 1. Umbilical cord blood testing paradigm. This diagram shows current minimum criteria testing on the left of the cord blood processing and use flowchart, whereas best practice criteria testing and new perspectives in UCB testing are shown on the right. Better practices involve processing UCB units to the MNC fraction and cryopreservation in this state. The TNC fraction is not a suitable starting material to implement best practices (Fig. 2). It also involves testing stem cell metabolic functionality and viability prior to cryopreservation to store the stem cell units predicted to have the highest quality. After cryopreservation, the first stem cell potency assay would be performed and the results uploaded to the cord blood inventory. When a UCB unit has been identified based on compatibility with the patient, a second potency assay would be performed to confirm the stability of the stem cells in the unit and to allow for its release. Both potency assays and perhaps one performed by the transplant center would provide predictive information regarding the UCB stem cell engraftment response. Assays to predict time to engraftment could still be performed but could not be used to determine potency. Abbreviations: CBB, cord blood bank; CFU, colony-forming unit; GM, granulocyte-macrophage; MNC, mononuclear cell; RBC, red blood cell; TNC, total nucleated cell; UCB, umbilical cord blood.
outcome [discussed in the Potency and Clinical Outcome section]. It is obvious that misleading assumptions have been introduced that have led to misinterpretation of what potency is and how it should be measured [37–40].

Potency and Clinical Outcome

One assumption is that potency must correlate with clinical outcome. This correlation is usually true for traditional drugs but is rarely the case for stem cell therapeutic products. Clinical outcome or, more specifically, the time for mature, functional cells to appear in the circulation after engraftment correlates to varying degrees with the TNC count and CD34 and CFU (GM) content [27, 41, 42]; however, for most cellular therapies, the clinical outcome occurs downstream of the intended response. The clinical outcome is the result of the intended response of engraftment by the “active” stem cell components [30], which then initiate the early reconstitution process. Clinical outcome is a retrospective indicator of stem cell potency but does not measure it. Potency is a prospective, quantitative measurement that predicts the correct dose to be used to ensure that the engraftment process does not fail because of low or lack of stem cell potency [18, 27]. Potency is used to release the product [30]. Stem cell potency, not TNC or viability, should be incorporated into the decision by the physician to use the unit for transplantation.

The TNC Fraction Cannot Be Used to Measure Quality or Potency

More advance technology has been used recently to test the hypothesis that the quality and potency of primitive stem cell populations can be determined using the TNC fraction in both UCB segments and units. This “null hypothesis” was tested using a standardized and validated [37, 43, 44] ATP bioluminescence stem cell proliferation assay and verified against the CFU assay [45]. The results decisively rejected this hypothesis and instead demonstrated that the mononuclear cell (MNC) fraction, not the TNC fraction, should be used because the latter not only masked but significantly underestimated the presence, quality, and potency of UCB stem cells in both segments and units [45]. This is illustrated in Figure 2, which shows an example of the difference between the TNC and MNC fractions for both the unit and associated segment. Another assumption negated by these studies was that dye exclusion viability provides a reliable method for demonstrating cell functionality. In fact, for both TNC and MNC fractions, dye exclusion viability did not correlate with metabolic viability and functionality [45]. As a result, cells may be viable using a dye exclusion method but may actually be dead metabolically. These and other results call into question present concepts and principles of UCB processing and testing. The question is, what must be changed to decrease risk and improve safety and efficacy for the patient and, at the same time, to become compliant with statutes and regulations?

NEW PERSPECTIVES

Cord blood banking, regardless of whether it is public or private, is a business operation. In the U.S., a UCB unit costs approximately $1,800 to collect, process, and store [46, 47]. To offset the costs, the U.S. government pays a public CBB approximately $1,100 for each UCB unit stored and inventoried, under the Stem Cell Thera- peutic and Research Act [47]. A CBB sells approximately 1%–2% of its inventory [46]; therefore, the cost of a single UCB to the patient or public health care provider is now between $40,000 and $60,000 [46]. To comply with FDA licensure requirements, the CBBs must invest approximately $1 million or more [49]. Despite these costs and investment, CBBs provide no assurance that their UCB stem cell products are of high quality and potency because testing does not include stem cells and is performed on the prefreeze sample. Because physicians now transplant UCB units based on the highest TNC count [26, 27], rather than on stem cell parameters, many of the units with low TNC counts will remain unused, producing extra costs for the CBBs. This is not a cost-effective business model, but that could change. It begins with the realization that if a patient is to receive a stem cell transplant, it follows that the stem cells in the product have to be measured because they are responsible for the success of the procedure. It also depends on whether regulatory agencies want to ensure patient safety and efficacy by requiring better practices in cord blood testing and the implementation of those practices by standards organizations and CBBs. Once these ideas are accepted, then the new perspectives summarized on the right side of Figure 1 can be applied.

Determining UCB Stem Cell Suitability for Storage and Potency Measurement

The first point to emphasize is that initial testing for UCB unit suitability and permanent storage should be performed and based on the postprocessed, prefreeze MNC fraction and not the TNC fraction. Automated processing systems can already accommodate this requirement. Although processing times and costs would be slightly increased, they could be offset by the increased number of stored cryopreserved MNC units. Prior to MNC freezing, the quality of the primitive stem cells would be assessed to determine whether the unit could be stored permanently. In addition, a minimum of four to six segments would be required, rather than the minimum required (two or three) [11]. Proper stem cell potency, however, would not be performed until after cryopreservation. This is because stem cell potency at the prefreeze stage will not represent the potency of the post-thaw unit. The results from the first potency assay (first segment) shortly after cryopreservation would be uploaded to the cord blood inventory, together with all other information concerning the unit. The NMDP EmTrax database now allows results from alternative, nonminimum testing to be included together with assay parameters, acceptance and rejection limits, and ranges. Such data are important to ensure those performing a UCB search that the unit is not only compatible with the patient but also that the stem cells have been analyzed to predict a positive engraftment response. Other cord blood inventories or registries should follow suit. Besides patient compatibility and other factors, transplantation should not be based solely on the number of cells but rather, more importantly, on the quality and potency of the stem cells.

Measuring Stem Cell Stability and Confirmatory Potency Testing Prior to Transplantation

This step would require a second potency assay to be performed (second segment) when a unit has been identified by the TC. The results would be used to release the unit for use. It is the responsibility of the CBB to provide a high-quality and potent stem cell product. This can be achieved only if the CBB performs a second assay and obtains similar results to the first potency assay prior to release and shipment, thus ensuring stability of the product [30]. Product stability is one of several reasons to perform a potency assay [30] because release of the product is also dependent on compatibility, microbial, endotoxin, mycoplasma, and other tests that ensure the patient’s safety.
Because new technology allows stem cell potency results to be obtained in 7 days or less time, there is usually sufficient time between preparing the patient for the transplant procedure and the arrival of the UCB unit at the TC. The reasons for performing a potency assay are (a) to provide the CBB with the assurance that their product is of high quality and can be released, (b) to give the transplant physician the confidence that the stem cells in the unit itself. A few TCs already perform this step but with minimum testing criteria [16]. A more purified product (MNC fraction) should produce lower CVs and better correlation between individual segments and the unit and thus greater reliability to accept and trust the results. It would also be expected that with potency characterization of the stem cells in the MNC fraction, improvements in the graft failure rate would occur and perhaps even a reduction in the length of time to engraftment (presently 20–24 days) [28, 29, 49].

To test the validity of the proposed model, a multicenter clinical trial was performed. The TC might use a third segment to confirm the quality and potency of the unit itself. A few TCs already perform this step but with minimum testing criteria [16]. A more purified product (MNC fraction) should produce lower CVs and better correlation between individual segments and the unit and thus greater reliability to accept and trust the results. It would also be expected that with potency characterization of the stem cells in the MNC fraction, improvements in the graft failure rate would occur and perhaps even a reduction in the length of time to engraftment (presently 20–24 days) [28, 29, 49].

To test the validity of the proposed model, a multicenter clinical trial would compare current practices with UCB unit purification and an MNC fraction and the proposed stem cell quality and potency testing with long-term follow-up.

Figure 3 shows the stem cell potency of 32-segment samples, of which 8 samples (25%) demonstrate a potency ratio for both stem cell populations below that of the reference standard, indicating their unsuitability for use. This could be interpreted as approximately corresponding to the highest graft failure rate of 24% [27–29]. A further 6 samples (18.75%) show that one or both of the stem cell populations exhibit a potency ratio similar to or slightly higher than the reference standard. This might be interpreted as being adequate for transplantation; however, as explained previously, both stem cell quality and potency need to be taken into consideration to release the product for use [37–40]. This allows the assay to demonstrate an accuracy to predict engraftment of more than 90% [38, 40]. It is possible that the samples tested in this study were of low quality and/or potency prior to or after initial processing. Such low-activity units might have been triaged either using the stem cell metabolic functionality and viability assay shown in Figure 1 or at the first potency assay step. Alternatively, it is also possible that stem cell potency of the sample may have deteriorated with time, which is the reason to perform a second or even a third potency assay. These considerations further emphasize the need not only for better practices but also for clinical trials to determine which UCB units might be unsuitable for use.

The concern about transplanting as many cells as possible has increased over the years [25, 26]. The greater the stem cell content, the higher the probability of engraftment potential because only a small fraction of the stem cells reach the bone marrow and engraft [50–52]; however, this should not deter using a unit that has higher purity and demonstrates greater quality and potency in the MNC fraction but with fewer cells to infuse. These suggestions and, indeed, radical changes must be openly debated and tested, but there are no scientific, moral, or ethical arguments not to improve UCB testing and include the checks and balances that should be associated with a clinical product. Unfortunately, the record of the cord blood community to test and adopt any change, let alone new technology that might reduce risk and improve patient safety and efficacy, is less than stellar. Problems defining a UCB unit as high quality using current practices were discussed at the ACBSCT meeting in December 2008 [53] and were still being debated in September 2014 [48].
Figure 3. The potency calculated for stem cell populations determined from umbilical cord blood (UCB) segment MNC fractions. Potency can be measured only by using a validated assay and the inclusion of a reference standard (RS) of the same material against which the sample can be compared. This comparison provides the measure of potency, the potency ratio, for each stem cell population (CFC-GEMM and HPP-SP). The potency ratio is calculated by the slope of the dose-response linear regression for each stem cell population from the segment sample divided by that for the RS. The UCB RSs were aliquots of cryopreserved MNCs from a single batch (lot) of processed umbilical cord blood. The potency of the RS is always 1. The potency ratios of both stem cell populations should be greater than 1 to provide assurance that the stem cells demonstrate sufficient engraftment potential when transplanted into the patient [37–40]. Abbreviations: CFC-GEMM, colony-forming cell-granulocyte, erythroid, macrophage, megakaryocyte; HPP-SP, high proliferative potential-stem and progenitor cell.

CONCLUSION

Grace Murray Hopper, a computer scientist and U.S. Navy rear admiral, stated that the most dangerous phrase in the English language is, “We’ve always done it this way” [55]. Such has been the dictum for UCB testing for the last 25 years. It is often argued that because hematopoietic stem cell transplantation works, there is no need for change. Hal Broxmeyer has stated that, “the future of cord blood transplantation will be bright as long as we do not become complacent and satisfied with the current status of the field and what is already known” [17]. Complacency will not increase the number of UCB units eligible for storage that exhibit stem cells of high quality and potency. It also will not reduce the graft failure rate and the length of time to engraftment. In the 21st century, failure to improve UCB testing and to implement best practices that include stem cell testing, without which there would not be a transplantation procedure, is a disservice and a betrayal of public trust.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

I.N.R. is chief executive officer and patent holder with HemoGenix.

REFERENCES

1. Buckner CD, Clift RA, Fefer A et al. Human marrow transplantation—current status. Prog Hematol 1973;8:299–324.
2. Haas R, Ho AD, Bredthauer U et al. Successful autologous transplantation of blood stem cells mobilized with recombinant human granulocyte-macrophage colony-stimulating factor. Exp Hematol 1990;18:94–98.
3. Körbling M, Holle R, Haas R et al. Autologous blood stem-cell transplantation in patients with advanced Hodgkin’s disease and prior radiation to the pelvic site. J Clin Oncol 1990;8:978–985.
4. Broxmeyer HE, Douglas GW, Hangoc G et al. Human umbilical cord blood as a potential source of transplantable hematopoietic stem/progenitor cells. Proc Natl Acad Sci USA 1989; 86:3828–3832.
5. Gluckman E, Broxmeyer HA, Auerbach AD et al. Hematopoietic reconstitution in a patient with Fanconi’s anemia by means of umbilical-cord blood from an HLA-identical sibling. N Engl J Med 1989;321:1174–1178.
6. Hematopoietic stem/progenitor cell products: Discussion of unrelated, allogeneic placental/umbilical cord blood and peripheral blood cell banking and transplantation. Available at http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/UCM187144.pdf. Accessed February 27, 2015.
7. Text of the Stem Cell Therapeutic and Research Act of 2005. https://www.govtrack.us/congress/bills/109/hr2520/text. Accessed February 27, 2015.
8. Guidance for industry: Minimally manipulated, unrelated allogeneic placental/umbilical cord blood intended for hematopoietic reconstitution for specified indications. Available at http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/UCM187144.pdf. Accessed February 27, 2015.
9. Guidance for industry: Biologics license applications for minimally manipulated, unrelated allogeneic placental/umbilical cord blood intended for hematopoietic and immunologic
reconstitution in patients with disorders affecting the hematopoietic system. Available at http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/UCM557135.pdf. Accessed February 27, 2015.

10 Standards for Cellular Therapy Product Services, 6th ed. Bethesda, MD: AABB, 2013.

11 Fifth edition NetCord-FACT international standards for cord blood collection, banking, and release for administration. Available at http://www.factweb.org/forms/store/ProductFormPublic/searchAction=1&Product_productNumber=627. Accessed February 27, 2015.

12 Petrini C. Umbilical cord blood banking: From personal donation to international public registries to global bioeconomy. J Blood Med 2014;5:87–97.

13 Ballen KK, Vertes F, Kurtzberg J. Umbilical cord blood donation: Public or private? Bone Marrow Transplant 2015 [Epub ahead of print].

14 Pasquini MC, Zhu X. Pasquini MC, Zhu X. Current uses and outcomes of hematopoietic stem cell transplantations:2014 CIBMTR summary slides. Available at http://www.cibmtr.org/pages/center slidereports/summaryslides/pages/index.aspx. Accessed February 27, 2015.

15 Butler MG, Menitove JE. Umbilical cord blood banking: An update. J Assist Reprod Genet 2011;28:669–676.

16 Barker JN, Byam C, Scaradavou A. How I treat: The selection and acquisition of unrelated umbilical cord blood grafts. Blood 2011;117:2332–2339.

17 Broxmeyer HE. Predicting the quality of transplantable cord blood collections through prefreeze and postthaw Agar scoring. Transfusion 2012;52:219–223.

18 Page KM, Zhang L, Mendizabal A et al. The Cord Blood Agar: A novel scoring system to optimize selection of banked cord blood grafts for transplantation (CME). Transfusion 2012;52:272–283.

19 Young W. Plasma-depleted versus red cell-reduced umbilical cord blood. Cell Transplant 2014;23:407–415.

20 Nakahata T, Okumura N. Cell surface antigen expression in human erythroid progenitors: Erythroid and megakaryocytic markers. Leuk Lymphoma 1994;13:401–409.

21 Meister B, Tötsch M, Mayr A et al. Identification of CD34+ cord blood cells and their subpopulations in preterm and term neonates using three-color flow cytometry. Biol Neonate 1994;66:272–279.

22 Balber AE. Concise review: Aldehyde dehydrogenase bright stem and progenitor cell populations from normal tissues: Characteristics, activities, and emerging uses in regenerative medicine. STEM CELLS 2011;29:570–575.

23 Lee HR, Shin S, Yoon JH et al. Aldehyde dehydrogenase-bright cells correlated with the colony-forming unit-granulocyte-macrophage assay of thawed cord blood units. Transfusion 2014;54:1871–1875.

24 Frändberg S, Boreström C, Li S et al. Exploring the heterogeneity of the hematopoietic stem and progenitor cell pool in cord blood: Simultaneous staining for side population, aldehyde dehydrogenase activity, and CD34 expression. Transfusion 2015;55:1283–1289.

25 Page KM, Mendizabal A, Betz-Stablein B et al. Optimizing donor selection for public cord blood banking: Influence of maternal, infant, and collection characteristics on cord blood unit quality. Transfusion 2014;54:340–352.

26 Bart T, Boo M, Babanova S et al. Impact of selection of cord blood units from the United States and Swiss registries on the cost of banking operations. Transfus Med Hemother 2013;40:14–20.

27 Page KM, Zhang L, Mendizabal A et al. Total colony-forming units are a strong, independent predictor of neutrophil and platelet engraftment after unrelated umbilical cord blood transplantation: A single-center analysis of 435 cord blood transplants. Biol Blood Marrow Transplant 2011;17:1362–1374.

28 Broxmeyer HE. Cord blood hematopoietic stem cell transplantation. Available at http://www.stemcell.org/node/693. Accessed February 27, 2015.

29 Allen D, Petraszko T, Elmoazzan H et al. A review of factors influencing the banking of collected umbilical cord blood units. Stem Cell Int 2013;463031.

30 Guidance for industry: Potency tests for cellular and gene therapy products. 2011. http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/UCM243392. pdf. Accessed February 27, 2015.

31 Guideline on potency testing of cell based immunotherapy medicinal products for the treatment of cancer. Available at http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003814. pdf. Accessed February 27, 2015.

32 NMDP current inventory requirements for new cord blood units. Available at https://network.bethematchclinical.org/WorkArea/DownloadAsset.aspx?id=5119. Accessed Febru ary 27, 2015.

33 Lamana M, Albella B, Rodríguez F et al. Conclusions of a national multicenter intercomparative study of in vitro cultures of human hematopoietic progenitors. Bone Marrow Transplant 1999;23:373–380.

34 Spellman S, Hurley CK, Brady C et al. Guidelines for the development and validation of new potency assays for the evaluation of umbilical cord blood. Cytotherapy 2011;13: 848–855.

35 Nawrot M, McKenna DH, Sumraid D et al. Interlaboratory assessment of a novel colony-forming unit assay: A multicenter study by the cellular team of Biomedical Excellence for Safer Transfusion (BEST) collaborative. Transfusion 2011;51:2001–2005.

36 Guidance for industry. Bioanalytical method validation. Available at http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070107.pdf. Accessed February 27, 2015.

37 Hall KM, Harper H, Rich IN. Hematopoietic stem cell potency for cellular therapy transplantation. In: Pelayo R, ed. Advances in Hematopoietic Stem Cell Research. Rijeka, Croatia: InTech; 2012.383–406.

38 Rich IN. Potency, proliferation and engrafement potential of stem cell therapeutics: The relationship between potency and clinical outcome for hematopoietic stem cell products. J Cell Sci Therap 2012;4:101–108.

39 Harper H, Rich IN. Measuring the potency of a stem cell therapeutic. Methods Mol Biol 2015;1235:33–40.

40 Rich IN. Tissue engineering: Propagation and potency evaluation. In: Atala A, Allickson J, Lindenmeier T, editors. Tissue engineering: Propagation and Engraftment Potential of Umbilical Cord Blood. Transfusion 2006;46:1803–1812.

41 Arovita T, Peramo K, Westman P et al. Associations among nucleated cell, CD34+ cell and colony-forming cell contents in cord blood units obtained through a standardized banking process. Vox Sang 2003;84:219–227.

42 Rich IN, Hall KM. Validation and development of a predictive paradigm for hematotoxicology using a multivariate biochemical luminiscence colony-forming proliferation assay. Toxicol Sci 2005;87:427–441.

43 Harper H, Rich IN. Stem cell predictive hematotoxicology. In: Alimoghaddam K, ed. Stem Cell Biology in Normal Life and Diseases. Rijeka, Croatia: Intech; 2013;79–107.

44 Patterson J, Moore CH, Salse J et al. Detecting primitive hematopoietic stem cells in total nucleated and mononuclear cell fractions from umbilical cord blood segments and units. J Transl Med 2015;13:94.

45 Bart T. Cost effectiveness of cord blood versus bone marrow and peripheral blood stem cells. Clinicoecon Outcomes Res 2010; 2:141–177.

46 National cord blood inventory: Practi-ces for increasing availability for transplants and related challenges. Available at http://bloodcell.transplant.hrsa.gov/cord/files/nationalcordbloodinventorygaoreport.pdf. Accessed February 27, 2015.

47 Advisory Council on Blood Stem Cell Transplantation: Monday, September 15, 2014. Available at http://bloodcell.transplant.hrsa.gov/about/advisory_council/meetings/2014%20September%20Meeting/meetingsummarynotes.pdf. Accessed February 27, 2015.

48 Ruggeri A, Labopin M, Sormani MP et al. Engraftment kinetics and graft failure after single umbilical cord blood transplantation using a myeloablative conditioning regimen. Haematologica 2014;99:1509–1515.

49 Stein J, Yaniv I, Askensay N. Critical early events in hematopoietic cell seeding and engraftment. Folia Histochim Cytobiol 2005;43: 191–195.

50 Pfeifferberger U, Yau T, Fink D et al. Asses-sment and refinement of intra-bone marrow transplantation in mice. Lab Anim 2015; 49:121–131.

51 Ishii M, Matsuoka Y, Sasaki Y et al. Development of a high-resolution purification method for precise functional characterization of primitive human cord blood-derived CD34-negative SCID-repopulating cells. Exp Hematol 2011;39:203.e1–213.e1.

52 Advisory Council on Blood Stem Cell Transplantation: December 15-16, 2008. Available at http://bloodcell.transplant.hrsa.gov/about/advisory_council/meetings/nov2014Meeting/2014Meetingminutesandnotes.pdf. Accessed February 27, 2015.

53 A plan for a cord blood association. Available at https://drive.google.com/file/d/0B5pOaOF3FUpWnJ30WWbTVZUanC/edit?pli=1. Accessed February 27, 2015.

54 Scheiber P. The wit and wisdom of Grace Hopper. The OCLC Newsletter 1987; 167. Available at: http://www.cs.yale.edu/homes/tap/Fil.es/hopper-wit.html. Accessed: June 29, 2015.