

Briefing Information – BRMAC February 27, 2002

Unrelated allogeneic hematopoietic stem/progenitor cells from placental/umbilical cord blood for hematopoietic reconstitution

Introduction

Health care professionals have had extensive clinical and non-clinical laboratory experience with placental/umbilical cord blood for hematopoietic reconstitution (UCB) since the first reported transplant of UCB in a child with Fanconi anemia in 1988. By 1993, large public repositories of UCB were established, in New York, Milan, and Dusseldorf¹. The functionality of long-term cryopreserved UCB cells was demonstrated by Broxmeyer et al. in their in vitro studies using CFU assays and, more recently, by the transplantation of CBU stored frozen for 15 years into NOD/SCID mice². A landmark clinical study of the efficacy of UCB as an alternative source of hematopoietic stem/progenitor cells (HSPCs), published by Rubinstein et al. in 1998, describes outcomes of a large cohort of recipients of UCB transplants from unrelated donors³. Over 2000 cord blood transplants have been performed worldwide, according to recent estimates. Many published reports support the use of UCB as an acceptable source of HSPCs for transplantation for selected recipients¹.

Currently, it is estimated that there are 70,000 cryopreserved, HLA-typed UCB products stored in cord banks worldwide¹. Many of the US establishments that collect, process, and store UCB products follow voluntary standards published by the AABB, FACT/NETCORD, and the NMDP. Despite the availability of published standards, the quality and characteristics of these stored products vary markedly due to several controllable and uncontrollable factors. First, the medical technology is rapidly evolving, resulting in the use of diverse collection and processing techniques at different facilities. Also, there is inherent variability in product volume due to uncontrollable donor factors. In addition, there is variability in the tests used for determining sterility and potency of the final product. Finally, the criteria for acceptability of a UCB product into an inventory vary among facilities, and the criteria for selection of a particular UCB product, such as extent of HLA match and total nucleated cell dose, are determined locally by transplant practitioners.

CBER issues for consideration

FDA seeks further advice and comments from the Advisory Committee about: (1) efficacy data derived from clinical studies of cord blood transplantation in recipients of particular age groups, and (2) assays for UCB potency; specifically, the utility of CD34+ cell count for predicting engraftment after cord blood transplant.

Efficacy of UCB from unrelated allogeneic donors for hematopoietic reconstitution

The major concerns of CBER, with respect to the use of UCB as an alternative source of HSPCs, are similar to those for HSPCs derived from peripheral blood (PB) or bone marrow (BM). They include: cell dose and how to measure it; safety, purity and potency of the product; donor safety; clinical outcome; and adverse reactions.

In addition, there are concerns and considerations that are particularly relevant to UCB. First, there are a limited number of HSPCs obtainable from a single donor. Thus, the dose of cells from one donor may be lower than necessary for engraftment. One consequence may be a higher rate of delayed engraftment or engraftment failure in adult recipients of UCB transplants. A balanced comparison to BM or PB is difficult due to confounding effects in much of the reported data, including different degrees of HLA disparity and differences in the disease entities and/or stage of disease being treated.

Second, UCB may have different properties or may contain different populations of primitive cells or lineages when compared with HSPCs derived from BM and PB⁴. There is suggestive evidence for a lower frequency of severe GVHD after UCB transplants, although direct controlled comparisons are not yet available. A major function of transplanted allogeneic HSPC products is to supplement intensive chemotherapy in removing tumor cells. This so-called graft versus leukemia/tumor effect is uncertain with UCB transplants.

Finally, all HPSC infusions provide a mixed population of early cells, some subsets of which may be relatively more mature and capable of providing cells for controlling infection rather than repopulating hematological lineages in a continuous manner. Once again there is little available data with which to evaluate different sources of HSPCs, with regard to subsets of true progenitor cells and more mature hematopoietic precursors.

Today we are focusing on the efficacy of UCB transplants from unrelated allogeneic donors and assays for potency testing of UCB products. The four questions posed to the committee and the advice being sought from the committee relate to these issues. The three invited guest speakers will address critical aspects of the efficacy of UCB and review updated UCB transplant experience.

CBER analysis of clinical outcome data

Data on clinical outcomes of UCB transplantation made available to CBER consisted of summary data. However, CBER recognized the importance of using primary data as the basis for analyzing clinical outcome. For this reason a series of analyses were performed by CBER staff on primary data provided by Drs

Pablo Rubinstein and Cladd Stevens of the NY Blood Center. This data was selected as the most suitable data to analyze because it was the most extensive.

There are multiple interacting factors that are considered in the selection of an optimal UCB product for transplantation and prediction of transplant outcomes. These factors include age and weight of the recipient, cell dose, HLA parity or disparity, among others. The following analyses focus on the age of the recipient and the number of cells transplanted as points of central interest. The results of our analysis are displayed in the four attachments to this briefing document.

Table 1 shows each of the four clinical outcomes which were examined; time (days) to achieve neutrophil engraftment, [> 500 Absolute Nucleated Cells /mm³], time (days) to achieve platelet engraftment [$>20,000$ platelets /mm³], incidence of severe acute Graft Versus Host Disease [aGVHD grade III and IV], and disease free survival. For purposes of analysis age ranges were grouped into three-year cohorts from newborn to age 29. There are sharp differences for all four outcomes between the youngest and oldest age groups. A stepwise analysis shows that the trend toward poorer outcomes becomes most marked in the middle range of cohorts for platelet engraftment and disease free survival, less so for ANC engraftment and even less clearly present for aGVHD.

Figure 1 graphically shows the same data. The numbers of patients in each cohort are shown below the abscissa.

Figure 2 shows disease free survival. It compares successive age cohorts. The most substantial difference between cohorts is between the cohort 10 (ages 9-11 years) and cohort 13 (ages 12-14 years). The numbers of transplants available for analysis were relatively lower and therefore less informative in the older age groups. Figure 1 shows the numbers of patients in each cohort.

Table 2 and figure 3 compare the 0-12 years to the 13-29 year old patients for the percentage achieving each outcome as well as the odds ratios. There is again a sharp difference in outcomes, with the older group having the poorer outcomes. Figure 3 shows disease free survival for the two groups compared above. The difference is statistically significant.

In the cord blood transplant setting the age-related outcomes are, in part, widely believed to be related to the number of nucleated cells in a single unit of cord blood, as mentioned earlier⁵. Adult recipients generally weigh more than children and the number of UCB nucleated cells expressed per kg body weight of recipient will be less in older recipients. Rubinstein et al. in their analysis of data on UCB transplants emphasize a close relationship between the number of cells and engraftment success³. BM and peripheral blood HSPC transplant outcomes also demonstrate age-related increases in transplant related events.

The results of CBER analyses can be interpreted as showing that:

(i) Older UCB recipients have a poorer outcome.

(ii) The poorer outcomes extend over the entire chronological age range of data but appear to be marked at or around ages 12-13.

Characterization and assays for potency testing of UCB

Although the only true measure of the potency of a HSPC graft is hematopoietic reconstitution in an ablated recipient, there are a variety of *in vitro* surrogates that have been used to attempt to predict engraftment potential. In order to be useful as clinical correlates, *in vitro* assessment of the viability, composition and function of these surrogates must be able to predict the product's capacity to engraft *in vivo*.

While human self-renewing pluripotential marrow-repopulating stem cells cannot be directly enumerated, their existence can be inferred by analysis of their progeny. Numerous studies in murine and canine models indicate a strong correlation between the transplanted dose of colony-forming progenitor cells and time to sustained hematopoietic reconstitution^{6;7}. Other reports from clinical trials in human subjects demonstrate a correlation between transplanted CFU-GM and days to neutrophil recovery⁸.

Complex *in vitro* systems for culturing progenitor cells at various levels of commitment have been developed to predict the type and number of cells necessary for engraftment. The earlier, less committed progenitors, such as cobblestone area-forming cells (CAFC), high proliferative potential colony-forming cells (HPP-CFC) and long term culture-initiating cells (LTC-IC) are present in low numbers and require long culture periods, so their utility is confined to experimental situations. As the level of commitment increases, the more committed progenitors such as CFU-GEMM, CFU-GM, and BFU-E are present in increasing numbers, with colonies apparent in 10 to 14 days. This may be enough time for evaluating products collected and stored for later infusion, but is not appropriate for assessing products collected for immediate transplantation or for monitoring circulating progenitor cell levels in peripheral blood of progenitor cell donors.

Furthermore, although there are data suggesting that the number of CFU-GM may be predictive of hematopoietic reconstitution, these culture assays are not standardized and the wide variety of colony shapes and sizes makes interpretation difficult and subjective. Because of these difficulties, correlation between colony number and days to engraftment varies widely in the published literature, suggesting the need for an alternative indicator of progenitor cell content for more accurate assessment of engraftment potential of progenitor cell grafts.

The purification of monoclonal antibodies to the CD34 antigen, a transmembrane glycoprotein present on a diverse population of multipotential and lineage-committed myeloid and lymphoid progenitors⁹, permitted the development of a dual-color direct immunofluorescence assay for rapid and reproducible enumeration of cells expressing this marker¹⁰. A positive correlation between total CD34+ cell count and circulating day-14 CFU-GM has been demonstrated¹¹, providing evidence that enumeration of CD34+ cells is a reliable indicator of progenitor cell content and, by extension, hematopoietic potential¹². Although the function of CD34 is not known, experimental evidence indicates a relationship with marrow localization and adhesion of progenitor cells. For optimal specificity in identifying and quantitating these rare cells (0.1 – 0.4% of cord blood cells) (cite), any immunophenotypic assay for CD34+ cells should include the use of a vital nucleic acid dye such as propidium iodide or 7-amino-actinomycin D (7-AAD)¹³. Addition of this parameter permits exclusion of dead cells and debris.

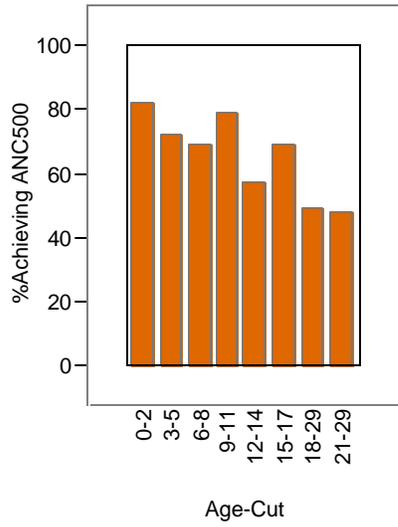
Extensive clinical experience demonstrates that the CD34+ cell content is an accurate predictor of neutrophil and platelet engraftment after myeloablative chemotherapy in recipients of peripheral blood progenitor cell transplants^{12; 14}. In addition, recent data from Wagner et al. from 102 patients receiving umbilical cord blood grafts identified CD34+ cell dose as the only factor among a number of variables that correlated with rate of engraftment¹⁵. We anticipate further advances in the available technology to assess the potency of hematopoietic stem/progenitor cell products using new methodologies and yet to be identified cell surface and bioactivity markers.

Table I

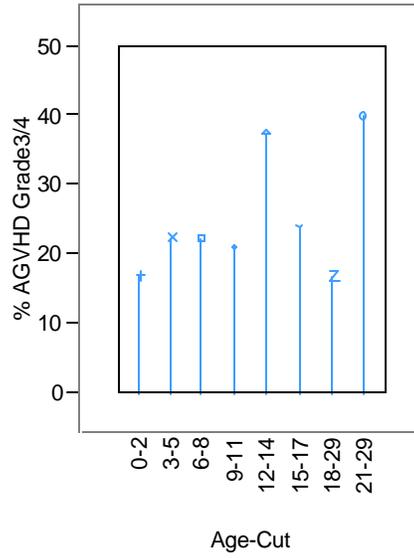
Clinical Outcomes for Different Age Groups

| Age Group | Achieving ANC 500 | Achieving Platelet 20,000 | AGVHD Grade 3/4 | Disease Free Survival |
|---------------------|--------------------------|----------------------------------|------------------------|------------------------------|
| (0, 1, 2) | 122/148 (82.4%) | 80/137 (58.4%) | 21/124 (16.9%) | 78/151 (51.7%) |
| (3, 4, 5) | 63/87 (72.4%) | 35/81 (43.2%) | 15/68 (22.1%) | 32/89 (36.0%) |
| (6, 7, 8) | 52/75 (69.3%) | 33/69 (47.8%) | 12/54 (22.2%) | 27/75 (36.0%) |
| (9, 10, 11) | 50/63 (79.4%) | 34/62 (54.8%) | 11/52 (21.2%) | 25/64 (39.1%) |
| (12, 13, 14) | 26/45 (57.8%) | 10/39 (25.6%) | 9/24 (37.5%) | 11/45 (24.4%) |
| (15, 16, 17) | 25/36 (69.4%) | 10/35 (28.6%) | 6/25 (24.0%) | 9/36 (25.0%) |
| (18, 19, 20) | 6/12 (50.0%) | 4/12 (33.3%) | 1/6 (16.7%) | 4/12 (33.3%) |
| (21-29) | 18/37 (48.7%) | 11/36 (30.6%) | 8/20 (40.0%) | 5/37 (13.5%) |
| Total | 362/503 | 217/471 | 83/373 | 191/509 |

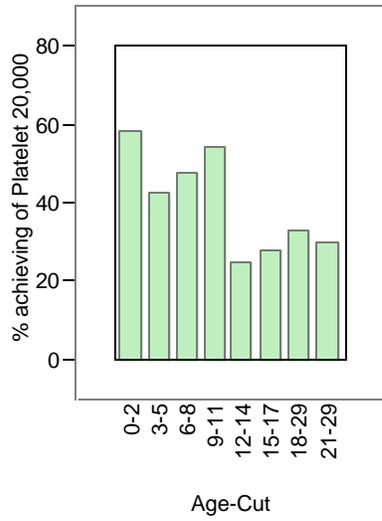
Figure 1. Clinical outcomes for different age groups



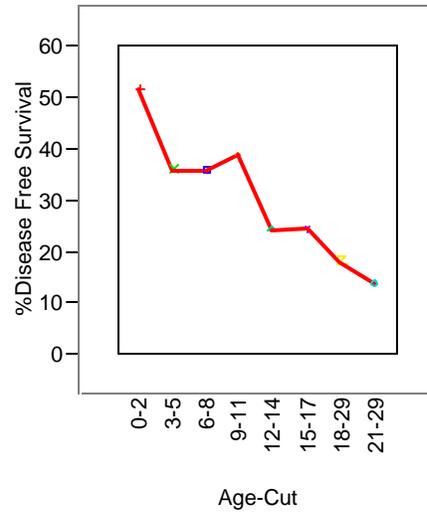
| N | 148 | 87 | 75 | 63 | 45 | 36 | 12 | 37 |



| 124 | 68 | 54 | 52 | 24 | 25 | 6 | 20 |

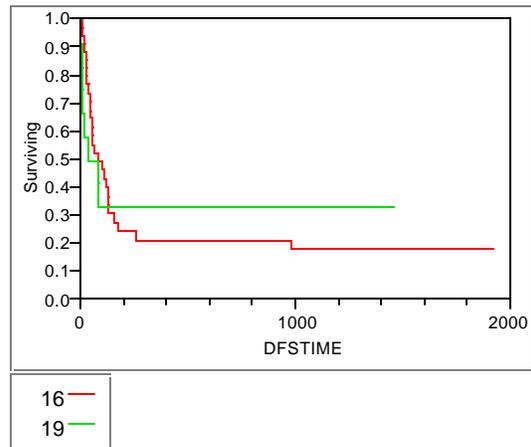
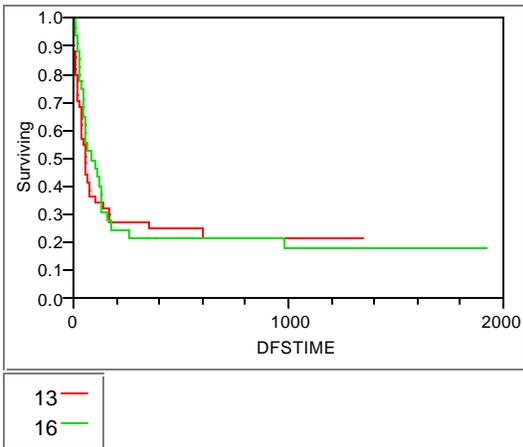
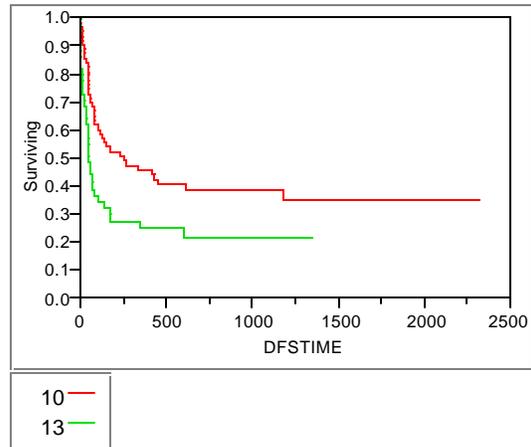
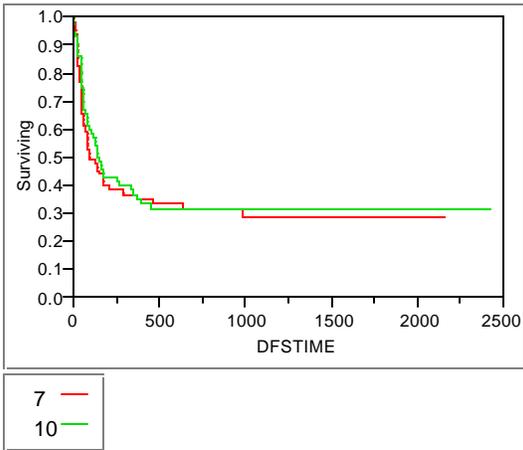
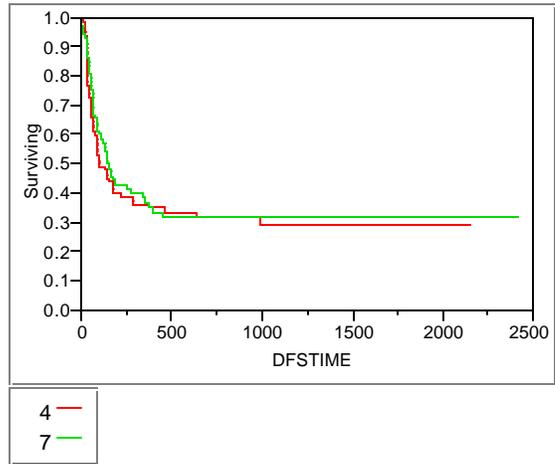
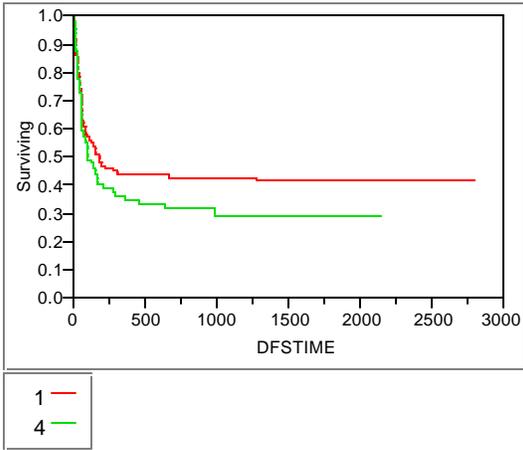


| N | 137 | 81 | 69 | 62 | 39 | 35 | 12 | 36 |



| 151 | 89 | 75 | 64 | 45 | 36 | 12 | 37 |

Figure 2. Disease Free Survival Curves for Two Consecutive Age Groups

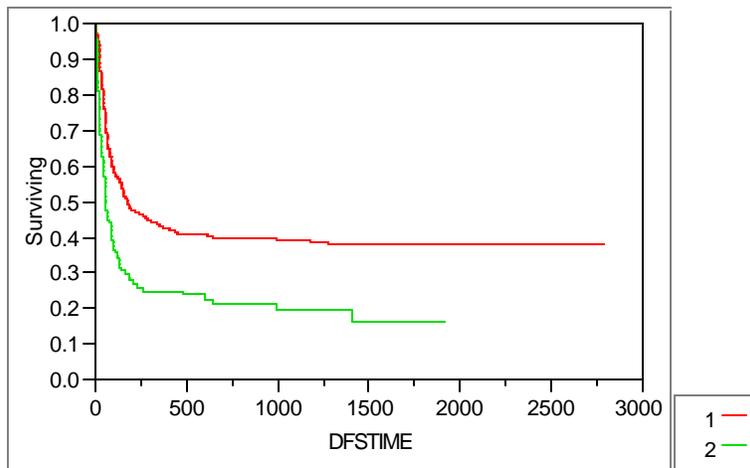


**1--Age 0-2; 4--Age 3-5; 7--Age 6-8; 10--Age 9-11;
13--Age 12-14; 16--Age 15-17; 19--Age 19-21.**

Table 2. Odds ratios for outcomes

| Outcomes | Proportions | | Odds Ratio* (Confidence Interval) *Age (13-29)/ Age (0-12) |
|------------------------------|-------------|-----------|--|
| | Age 0-12 | Age 13-29 | |
| <i>ANC 500</i> | 76.6% | 56.1% | 0.39 (0.25, 0.61) |
| <i>Platelet 20,000</i> | 51.4% | 28.0% | 0.37 (0.23, 0.59) |
| <i>AGVHD grade 3/4</i> | 20.5% | 30.8% | 1.73 (0.95, 3.13) |
| <i>Disease Free Survival</i> | 41.8% | 22.8% | 0.41 (0.25, 0.67) |

Figure 3. Survival plot for younger and older age groups
(Age-Cut include age 12)



** 1-- Age 0-12; 2-- Age 13-29

Tests Between Groups

| Test | ChiSquare | DF | Prob>ChiSq |
|----------|-----------|----|------------|
| Log-Rank | 24.76 | 1 | <.0001 |
| Wilcoxon | 26.95 | 1 | <.0001 |