

# ADVANCES IN HEMATOLOGY

Current Developments in the Management of Hematologic Disorders

Section Editor: Craig M. Kessler, MD

## Mesenchymal Stromal Cell Expansion for Cord-Blood Engraftment



Marcos de Lima, MD  
 Medical Director  
 Hematologic Malignancies and Stem Cell Transplant Program  
 University Hospitals Seidman Cancer Center  
 UH Case Medical Center  
 Cleveland, Ohio

### **H&O** How is umbilical-cord-blood transplantation used in patients with hematologic cancers?

**ML** Umbilical-cord-blood transplantation has become an established source of hematopoietic stem cells for transplant in malignant and nonmalignant indications, particularly among pediatric patients. In adults, it is used somewhat less often because of the relatively limited amount of cord-blood stem cells available. The net result is that it is easier to engraft smaller bodies (on a per kilogram basis) given the relative stem cell dose.

### **H&O** What are the benefits of this approach?

**ML** The main drive to use cord blood is a reduced risk of graft-versus-host disease. On a match-by-match comparison between donor and recipient, graft-versus-host disease is less common with stem cells sourced from cord blood than bone marrow or peripheral blood. The reduced incidence of graft-versus-host disease has allowed us to increase the donor pool by expanding the degree and definition of “acceptable” matching. By accepting more mismatches, it is possible to find donors for patients who would normally lack an excellent or “perfect” match. Another advantage is availability. Cord blood is the only source of stem cells that is banked, so the procurement is fast.

### **H&O** Are there limitations to the use of cord-blood transplantation?

**ML** Cord-blood transplantation is associated with more graft failures due to the limited amount of cells. Another potential limitation is delayed time to engraftment, leading to complications such as longer hospital stays, longer

dependence on transfusions, and possibility of infections. On a match-by-match basis, cord-blood transplantation has also been associated with delayed immune recovery, especially in adults, even if the blood counts have recovered. Patients may experience infectious complications for a significant amount of time after the transplant.

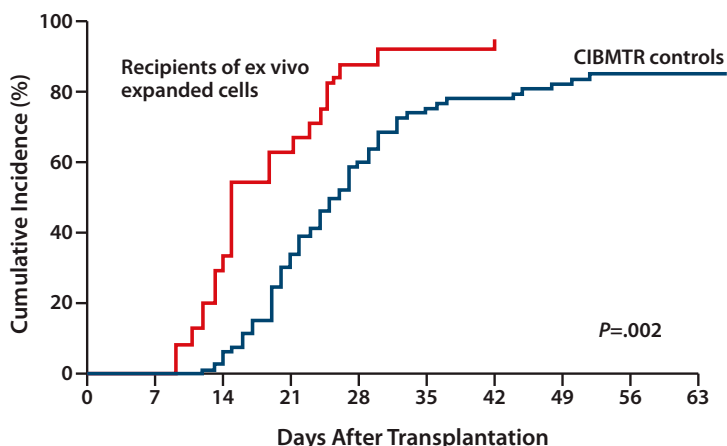
### **H&O** What prompted your recent study on cord-blood transplantation?

**ML** Cord-blood transplantation is often associated with a long time to neutrophil and platelet engraftment. For years, Dr. Elizabeth Shpall, the senior author of the article, has been investigating ex vivo expansion of stem cells in the laboratory before administration to patients, in collaboration with John McMannis and Ian McNiece. There was laboratory evidence to suggest that the addition of mesenchymal stromal cells to the ex vivo expansion platform already in use (in a liquid culture system) seemed to make the expansion much better. We hypothesized that expansion of stem cells with mesenchymal stromal cells could provide a more consistent and reliable platform of expansion.

### **H&O** Could you please describe the study design?

**ML** It was a phase II study that was intended to evaluate the efficacy and safety of the mesenchymal stromal cell expansion technique. The 2-week culture is a liquid culture system that contains growth factors for cells in a feeding media. Before the cord-blood cells were put into this media, we introduced mesenchymal stromal cells to the culture bags. Mesenchymal stromal cells by nature tend to adhere to plastic. Then we put the cord-blood cells into the same media. In a very broad statement,

**A Neutrophil Engraftment**



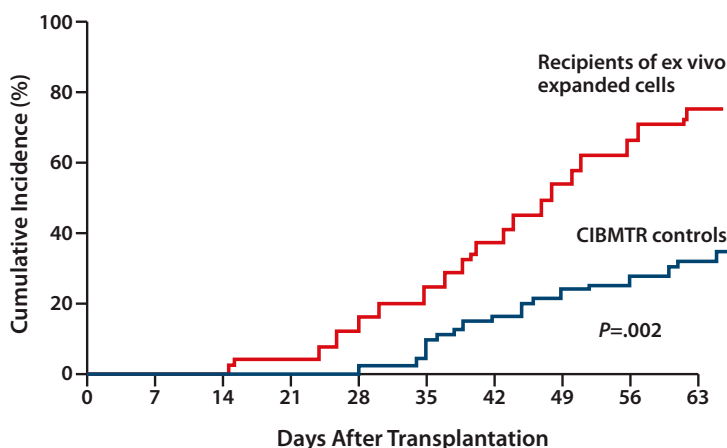
**CIBMTR Controls**

No.	80	80	74	48	25	12	10	5	3	1
No. with engraftment	0	0	5	22	21	12	2	3	2	0

**Recipients of Ex Vivo Expanded Cells**

No.	24	24	16	8	3	1	0	0	0	0
No. with engraftment	0	0	8	8	5	1	1	0	0	0

**B Platelet Engraftment**



**CIBMTR Controls**

No.	79	79	78	73	70	63	57	48	42	33
No. with engraftment	0	0	0	1	1	6	5	6	3	4

**Recipients of Ex Vivo Expanded Cells**

No.	24	24	24	23	20	17	14	10	6	4
No. with engraftment	0	0	0	1	3	2	3	4	3	2

**Figure 1.** Engraftment of neutrophils and platelets. A) At 26 days, the cumulative incidence of neutrophil recovery was 88% among recipients of expanded cord blood and 53% among CIBMTR controls. B) At 60 days, the cumulative incidence of platelet recovery was 71% among recipients of expanded cord blood and 31% among CIBMTR controls. Data regarding platelet engraftment were not available for 1 patient in the CIBMTR control arm. CIBMTR=Center for International Blood and Marrow Transplant Research. From the *New England Journal of Medicine*. de Lima M, McNiece I, Robinson SN, et al. Cord-blood engraftment with ex vivo mesenchymal-cell coculture. Volume 367:2305-2315. Copyright © 2012, Massachusetts Medical Society. Reprinted with permission from the Massachusetts Medical Society.

one could say that we were mimicking what these cells do in the bone marrow. Mesenchymal stromal cells are thought to “talk” to bone marrow cells, potentially feed-

ing them or giving them signals to live and prosper.

At the end of 2 weeks, patients received these cells for transplant. To provide a safety net, patients also received

a cord-blood unit that was not expanded. This study was not randomized; data were compared with those from historic controls obtained from an international registry (including patients that received a double cord-blood transplant, without expansion).

### H&O What were the study results?

**ML** We initially noticed a faster-than-expected neutrophil engraftment (Figure 1). The CD34-positive cells were expanded by a median factor of approximately 30 times. The time to neutrophil engraftment decreased to a median of approximately 15 days, a better outcome than the median of 25 days observed in historic controls from the international registry. We also saw a very consistent platelet engraftment, which was not necessarily faster than usual but was more consistent. Production of platelets can be a problem with cord-blood transplant in adults. Sometimes recipients take a long time to adequately produce platelets. A significant proportion of patients do not produce platelets, which ties them to transfusions for a long time. Inconsistent platelet engraftment is not necessarily as life-threatening as lack of new neutrophils, but it is associated with potential for bleeding and quality of life issues.

The trial was performed at a single institution, and findings must be confirmed in a multicenter, randomized study. My colleagues and I are now undertaking an international phase III trial, supported by Mesoblast, the company that provides the mesenchymal stromal cells. This study will follow the same design: one arm will receive 2 cord-blood units that are not expanded (the control group), and the other arm will receive 1 unit that was expanded together with 1 unit that was not expanded. We hope that the phase III trial will show that the expanded cord-blood cohort achieves faster neutrophil engraftment and faster—or at least more reliable—engraftment of platelets.

### H&O What was observed about the engraftment?

**ML** The expanded cord-blood unit grew fast and was responsible for the early engraftment. Meanwhile, the unexpanded blood grew slowly but surely in its own time, while at some point, the expanded cells burnt out and disappeared. Ultimately, it was the unexpanded blood unit that assured long-term engraftment. The mechanisms behind this process are unknown. It may be that this approach pushed these stem cells toward differentiation, and they lost their capacity to be stem cells and died a few weeks after transplantation but not before they were able to provide early protection to the patient. Another potential explanation for this finding is that the culture system depleted lymphocytes. These lymphocytes were probably part of the fighting army that normally helps donor cells engraft, and

this lack of lymphocytes favored the long-term engraftment of unexpanded cells. It is possible that lymphocytes eradicated the expanded cells in a graft-versus-graft fight, and the unexpanded cells remained.

Although the majority of the patients had evidence of only long-term engraftment by the unexpanded cord-blood unit, the expanded cells remained in a minority of patients. There are now a few patients with blood made from the 2 donors, with persistent mixed chimerism more than a year after transplant.

### H&O Does coculture with mesenchymal stromal cells require more time and/or resources?

**ML** The 2-week culture time is an extra requirement. A randomized trial would be needed to show whether this extra step is justified by a better outcome, such as faster engraftment, fewer transfusions, fewer infections, and better survival.

### H&O Is there any other promising research in this area?

**ML** Dr. Colleen S. Delaney and colleagues at the Fred Hutchinson Cancer Research Center are growing cells in the laboratory using an engineered notch ligand, and the recipients of transplants so treated are also engrafting faster than in the historic experience. Large clinical trials are currently evaluating another technique that utilizes a copper chelator to grow more cells. A substance, tetraethylenepentamine, is introduced into the expansion media that depletes copper, and this intervention appears to make the cells less likely to differentiate and allows expansion of early progenitors in a liquid culture system. Dr. Elizabeth Shpall, my colleague at MD Anderson, is exploring a technology called fucosylation, in which sugar molecules are added to the cells before they are infused in the patient, with the goal that the cells will be more likely to find their niche in the bone marrow, and therefore improve engraftment efficiency. It is hoped that this process will expedite engraftment, not by sheer numbers, but by making the cells smarter. Clinical trials are beginning.

### Suggested Readings

Delaney C, Bollard CM, Shpall EJ. Cord blood graft engineering. *Biol Blood Marrow Transplant.* 2013;19(suppl 1):S74-S78.

Delaney C, Heimfeld S, Brashem-Stein C, Voorhies H, Manger RL, Bernstein ID. Notch-mediated expansion of human cord blood progenitor cells capable of rapid myeloid reconstitution. *Nat Med.* 2010;16:232-236.

de Lima M, McMannis J, Gee A, et al. Transplantation of ex vivo expanded cord blood cells using the copper chelator tetraethylenepentamine (TEPA): a phase I trial. *Bone Marrow Transpl.* 2008;41:771-778.

de Lima M, McNiece I, Robinson SN, et al. Cord-blood engraftment with ex vivo mesenchymal-cell coculture. *N Engl J Med.* 2012;367:2305-2315.

Oran B, Shpall E. Umbilical cord blood transplantation: a maturing technology. *Hematology Am Soc Hematol Educ Program.* 2012;2012:215-222.