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Ex vivo expansion of umbilical cord blood hemopoietic stem and progenitor cells

Since the first unrelated donor umbilical cord blood transplant (UCBT) in July 1993, more than 3500 UCBT have been performed worldwide for the treatment of various malignant and nonmalignant disorders [1]. At the end of the first decade of unrelated UCBT, the clinical results clearly indicate the critical importance of cell dose not only on the speed of neutrophil and platelet recovery and incidence of engraftment but also on the incidence of nonrelapse mortality and probability of disease-free survival [1–4]. Further, the data suggest the possibility that higher cell dose may partially compensate for the deleterious affect of HLA disparity on survival [4]. Together the results of these analyses incite us to develop new strategies for compensating for the limited number of hematopoietic stem and progenitor cells that can be collected from a single placenta, as cell dose represents the single most important obstacle for the successful use of UCBT.

If the premise is accepted that UCBT is limited by cell dose, the first question is what cells need to be expanded. Although there are considerable data that correlate CD34 and CFU-GM cell doses with rate of hematopoietic recovery and incidence of engraftment after UCBT, it is realized that these are surrogates for more primitive stem and progenitor cells. Thus, it is unknown which cell should be expanded (i.e., stem cell, primitive progenitor, or more committed progenitor). Most would agree that expansion of terminally differentiated cells would be of little benefit. Further, most would agree that maintenance or near-maintenance of the stem cell pool within the expanded fraction would be optimal (i.e., if not expanded, stem cells should not be lost at the expense of generating progenitors incapable of self-renewal).

As it is unknown which progenitor population will contribute to an earlier “wave” of neutrophil recovery that would contribute to more rapid hematopoietic recovery after UCBT,

there are reasons for attempting to expand the most primitive progenitors detectable by readily available assays—NOD-SCID repopulating cells (SRC) and long-term culture-initiating cells (LTC-IC); these include: (1) reducing the period of neutropenia after transplantation, and (2) improving transduction by retroviral vectors.

Using a combination of cytokines (e.g., stem cell factor, FLT3L, thrombopoietin), chemokines (IL-8, MIP1a, VEGF), and O-sulfated heparin to mimic stroma-conditioned media supplemented with high-dose cytokines in the absence of serum, Verfaillie et al. [5–7] have demonstrated 25-fold expansion of CFU-GM, 1.3-fold expansion of LTC-IC, and maintenance of SRC. Notably, more recent data suggests that expansion of SRC with the addition of all-trans-retinoic acid can be achieved, but only in the presences of stroma, suggesting an indirect effect on the hematopoietic stem cell population (Verfaillie, unpublished data). While intriguing, the later approach is of no immediate clinical value due to the requirement of a genetically modified stromal cell line.

The GMP production of a stromal-like media requires a mix of various cytokines, chemokines, and a glycoaminoglycan. Most of these reagents are not produced and will never be produced in clinical grade. As it is unlikely that any expansion culture condition exists in which all reagents are produced with clinical-grade components, GLP-grade materials must be used. It remains to be determined whether the FDA will allow the clinical application of this approach.

However, making the presumption that the FDA will allow the clinical testing of an ex vivo expansion culture composed of GLP-grade reagents, it is critical that the manufacture of the expansion culture media be performed in a GMP cell therapy facility staffed with technologists experienced in clinical cell therapy and the complex requirements of a GMP quality assurance program. This requirement is not only to minimize the risks to human subjects but also to reduce the chance of protocol deviations and optimize the identification of manufacturing problems and process improvements.

Importantly, it has been difficult to determine how expansion culture might be tested clinically. Initially, it must be presumed that expansion culture may be harmful to the hematopoietic stem cell pool, thus increasing the risk of graft failure. For this reason, all prior studies have placed only a proportion of the UCB graft into expansion culture. As most grafts have been stored in one cryobag, however, this requires that the expansion culture begin on the day of transplant with its infusion 10 to 14 days later. The approach is problematic: 1) it reduces the ability to detect any positive impact on speed of neutrophil recovery since it is infused weeks after the initial transplant, and 2) absence of a genetic marker prevents any ability to track the expanded population and to determine whether expansion culture is toxic to the stem cell pool as measured by long-term engraftment. Therefore, our group has explored the use of transplanting two partially HLA-matched UCB units as a

model for testing the safety and potential efficacy of expansion culture. Preliminary studies [8,9] suggest that this approach may indeed serve to be a useful model as it provides a measure of safety (by the infusion of an unexpanded product) and a means of tracking the progeny of the expanded product (by measuring molecular markers that distinguish donor 1 from donor 2 from host). Studies are underway testing both the model and expansion culture.

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