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Reviews

Double Umbilical Cord Blood Transplantation: Relevance of Persistent Mixed-Unit Chimerism

Hasan Hashem [1], Hillard M. Lazarus [2,*]

[1] Department of Pediatrics, Case Western Reserve University, Cleveland, Ohio
[2] Department of Medicine, Case Western Reserve University, Cleveland, Ohio

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A B S T R A C T

Double umbilical cord blood transplantation (UCBT) was developed as a strategy to circumvent the cell dose limitation of single UCBT with a concomitant potential benefit of lowering the rate of leukemia relapse. Sustained hematopoiesis after double UCBT usually is derived from a single donor unit, as only a few patients have been reported to display stable mixed-unit chimerism for varying periods of time. Explanations for the 1 unit dominance, predictors for identifying unit superiority, and persistence of long-term mixed-unit chimerism remain elusive. Review of published literature revealed only 11 of 280 patients (4%) with mixed-unit chimerism for at least 1 year after transplantation, with 3 patients receiving reduced-intensity conditioning regimens. Mixed-unit chimerism was more likely if both units were closely HLA matched to each other. Outcome data for patients with stable mixed-unit chimerism, for the most part, were scarcely reported. Analysis of the small sample size revealed a potential advantage of stable mixed-unit chimerism on enhancing the graft-versus-leukemia effect; however, definitive conclusions cannot be made on the effect of mixed-unit chimerism on the rates of graft-versus-host disease. Therefore, gathering outcome data prospectively in larger clinical series will help answer the question of whether stable mixed-unit chimerism is either beneficial and, therefore, should be strived for, detrimental and, thus, needs to be eliminated, or if it is of no clinical consequence.

INTRODUCTION

Hematopoietic progenitor cells isolated from umbilical cord blood (UCB) are an alternative graft source for allogeneic hematopoietic cell transplantation (AlloHCT) for those patients lacking a suitable histo-identical sibling or a well-matched adult unrelated donor. UCB has the advantage of being readily available with a less stringent requirement for human HLA matching because of the immunologic naiveté of UCB cells and the reduced numbers of lymphocytes in the unit [1,2]. This graft source has become a standard therapeutic option for pediatric hematologic malignancy patients and the result of using UCB compares favorably with unrelated blood and bone marrow grafts for AlloHCT [3]. In larger children and adults, however, UCB transplantation (UCBT) efficacy is severely limited by the low progenitor cell dose per recipient weight, leading to high risk of delayed or failure of engraftment [4].

Various strategies to overcome this drawback include the use of double (ie, dUCBT) rather than single-unit UCBT, ex vivo expansion of UCB units, direct intrabone marrow injection, and use of agents to enhance cell homing [5-8]. These interventions have been met with limited success. Despite over a decade of using these approaches, no single technology has emerged as a preferred approach. In the vast majority of dUCBT cases, sustained engraftment of only 1 donor unit ultimately dominates and the other unit no longer can be detected [9,10]. In rare cases, long-term hematopoiesis can be observed from both donor units in varying ratios, a condition referred to as *mixed-unit chimerism*. To date, no factors have been identified in these cases that reliably predict which unit will emerge as the dominant unit. The mechanism for such single-unit dominance remains to be elucidated [9,10]. The study of dUCBT is of even greater interest given recent reports suggesting that dUCBT may be associated with a reduced risk of leukemia relapse, which is thought to possibly be a result of unit-to-unit allogeneic interactions [11,12].

Chimerism results after transplantation are significant. In patients with malignant diseases, chimerism is primarily used to detect early disease relapse, but it can also indicate

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* Correspondence and reprint requests: Hillard M. Lazarus, MD, FACP, Department of Medicine, University Hospitals Case Medical Center, 11100 Euclid Avenue, Cleveland, OH 44106.
E-mail address: hillard.lazarus@case.edu (H.M. Lazarus).

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Table 1
Clinical Studies Addressing Single-Unit Chimerism

Author, yr	Patient, n	Age, Median (Range), yr	Disease	Regimen	TNC Dose, $\times 10^7$/kg	CD34 dose, $\times 10^5$/kg	Chimerism Pattern	Comment
Barker, 2005 [14]	23	24 (13-53)	ALL, AML, CML	MAC	Dominant: 1.8 Non-dom: 1.9	Dominant: 1.5 Non-dom: 2.5	1 unit dominates in all patients by d 100	Higher $CD3^+$ dose was significantly associated with SUD (P value <.01); however, differences in $CD3^+$ cell dose were minimal (.6 $\times$ 10^7 versus .4 $\times$ 10^7)
Kang, 2006 [17]	8	14 (6-17)	AML, ALL, SAA	MAC	4.68	1.75	SUD by d +28 in all patients	Selection of 1 strong unit (with high numbers of total nucleated and/or $CD3^+$ cells) and 1 weak unit to activate strong 1; thus creates SUD, might result in better outcome
Ballen, 2007 [15]	21	49 (24-63)	AML, NHL, CLL, MDS, ALL	RIC	Dominant: 2.2 Non-dom: 1.8	Dominant: 1.8 Non-dom: .7	Dominance of 1 UCB unit at 3 mo in all patients	First unit infused more often dominant; 90% of the units infused 4 hr apart
Brunstein, 2007 [16]	93	51 (17-69)	ALL, AML, MDS, NHL, CML, HL	RIC: 1/3 given ATG	3.7	4.9	Early transient MUC followed by SUD at d 100	No factors reliably predict which of 2 UCB units predominate
Yin, 2011 [21]	12	31 (17-42)	AML, ALL, CML	MAC, all given ATG	Dominant: 2.85 Non-dom: 2.5	Dominant: .55 Non-dom: .25	SUD in all patients at 1 yr	Neither TNC nor $CD34^+$ cell doses correlated with dominance
Wallet, 2013 [22]	136	38.5 (9-63)	ALL, AML, CML, CLL, NHL, MM	MAC: one third RIC: two thirds, one quarter given ATG	3.1	1.4	Unit gender-matched with recipient more likely to become dominant	Disease status at time of transplant remains the major prognostic factor for outcome
Song, 2013 [23]	29	23 (10-48)	AML, CML, ALL, MDS	MAC: one fifth given ATG	4.7	2.4	1 transient MUC for short time	Neither TNC nor $CD34^+$, $CD3^+$ or GM-CFU affected unit dominance
Kai, 2013 [24]	61	37 (10-54)	ALL, AML, CML, MDS, NHL	MAC	3.53	1.04	All patients had SUD by d +60	Only degree of HLA disparity in host-versus-graft direction affected SUD

TNC indicates total nucleated cell; ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; Non-dom, nonpredominant unit; SUD, single-unit dominance; SAA, severe aplastic anemia; NHL, non-Hodgkin lymphoma; CLL, chronic lymphocytic leukemia; MDS, myelodysplastic syndrome; HL, Hodgkin lymphoma; MUC, mixed-unit chimerism, MM, multiple myeloma.

impending rejection. In patients with nonmalignant disorders, it is merely used to monitor successful engraftment. The ability to detect the dominant unit early after transplantation might be useful clinically, as delayed hematopoietic recovery and immune reconstitution after dUCBT remain ongoing limitations to more widespread adoption of the approach using UCB as a donor graft source. Moreover, lack of available donor lymphocyte infusion in UCBT (as potential adoptive immunotherapy to improve engraftment or to treat relapse) is problematic. Here, we review the dUCBT literature regarding the frequency of mixed-unit chimerism occurrence, the clinical outcomes, and implications for UCB graft selection.

METHODS

We undertook a PubMed literature search for relevant clinical trials and reviews (from January 1, 1985 to April 1, 2014) using the following key words: umbilical cord blood, transplantation, double, mixed chimerism, and dominance. We used those key words in different combinations. We focused on the studies that are related to our review subject of dominance and mixed-unit chimerism in the setting of dUCBT. We also cross-referenced review articles but focused on clinical studies and some preclinical trials, regardless of patient ages and minimum numbers of patients in a trial or report.

After successful AlloHCT, the recipient usually adopts the donor hematopoietic system and becomes a full donor chimera. In some cases, however, recipient hematopoietic cells remain and the patient instead becomes a mixed chimera. *Split chimerism* is used when the coexistence of donor and recipient cells is observed in specific cellular lineage but not in others. In the current article, we focused on hematopoietic progenitor cell chimerism. A patient is considered to be *a mixed chimera* if he or she has 5% to 95% of hematopoietic cells of donor origin [13]. After a period of transient engraftment of both UCB units, a single unit emerges as the "winner" to sustain long-term hematopoiesis, ie, at least 90% marrow reconstitution by donor cells [14-16]. The time frame for determining dominance has not yet been clarified [17]. Usually, by day 21 after transplantation, single-unit dominance can be detected in over 80% of patients, although dominance as soon as 14 days after transplantation has been reported [10,18]. Sustained detection of both UCB units in varying ratios over 21 days generally is termed *mixed-unit chimerism*. Dominance reversion occurs when the fraction of cells of the predominating UCB unit decline gradually and give up dominance to the other unit in the state of mixed-unit chimerism. In an analysis of 23 dUCBT after myeloablative conditioning (MAC), hematopoiesis was observed from a single donor in 76% patients at day 21 and in 100% by day 100 after transplantation [14]. Likewise, a review of 81 dUCBT after a nonmyeloablative (NMA) regimen, single-donor chimerism was detectable in 57%, 81%, and 100% of patients at day 21, 100, and 365, respectively [16].

Chimerism is often determined using bone marrow or blood samples obtained at 21, 60, 100, 180, 360, and 720 days after transplantation, with the use of additional time points as clinically indicated. Methods and approaches for chimerism monitoring after dUCBT are discussed in detail by Kristt et al. [19].

SINGLE-UNIT DOMINANCE

Although single-unit dominance has been well described, prior studies have not identified the mechanism or a reliable method of predicting which will be the long-term engrafting unit [9,10]. Verneris et al. stated "predicting the winning unit seemed impossible and more like atmospheric noise" [20]. However, other investigators continue to attempt to define predictors of UCB unit dominance. Gutman et al. showed evidence that donor T cells from the engrafting UCB unit specifically recognize the nonengrafting unit [18]. Taking into consideration the intrinsic properties of the 2 infused units and the immune interactions between the recipient and the donor units, some studies have attempted to assess whether single-unit dominance is influenced by the intrinsic features of the UCB units. Table 1 depicts 8 clinical reports addressing single-unit chimerism. The studies contained 8 to 136 patients; the majority of subjects received MAC regimens and the most frequent disease indication was acute