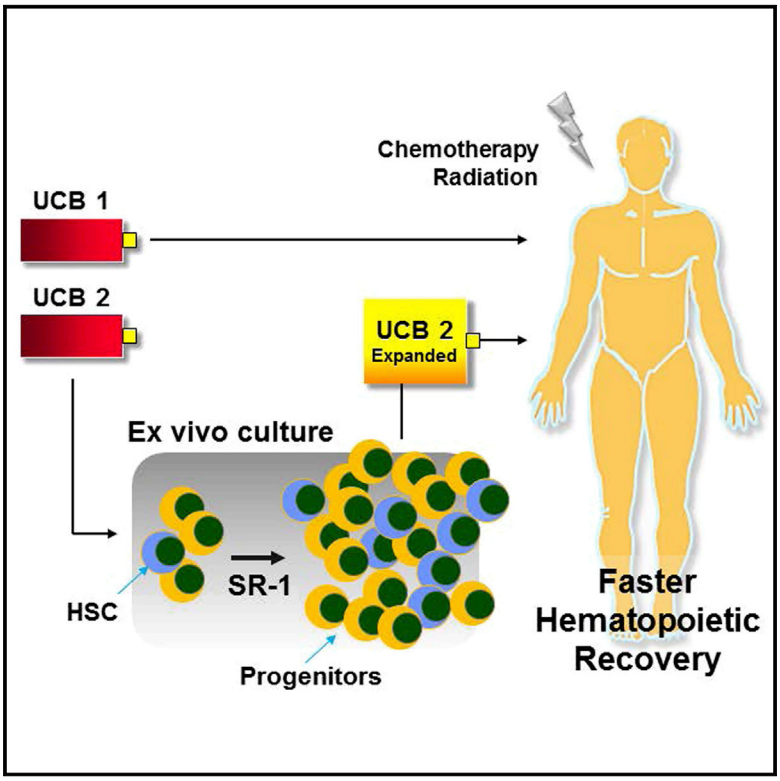
Clinical Progress

Cell Stem Cell

Phase I/II Trial of StemRegenin-1 Expanded Umbilical Cord Blood Hematopoietic Stem Cells Supports Testing as a Stand-Alone Graft

Graphical Abstract



Authors

John E. Wagner, Jr., Claudio G. Brunstein, Anthony E. Boitano, ..., Julie Jones, Michael P. Cooke, Conrad C. Bleul

Correspondence

wagne002@umn.edu

In Brief

Clinical testing of the aryl hydrocarbon antagonist StemRegenin-1 showed robust expansion of hematopoietic stem and progenitor cells and an adequate safety profile in the setting of double UCB transplant, supporting its further testing for safety and efficacy as a stand-alone graft after myeloablative conditioning.

Highlights

- SR-1 led to significant expansion of CD34+ HSPCs in culture
- Seventeen patients with hematological malignancy received SR-1 expanded UCB
- SR-1 expanded cells were co-infused with a second unexpanded UCB unit
- SR-1 expansion improved neutrophil and platelet recovery compared to controls

Wagner et al., 2016, Cell Stem Cell *18*, 144–155
January 7, 2016
http://dx.doi.org/10.1016/j.stem.2015.10.004

CellPress

Cell Stem Cell
Clinical Progress

Phase I/II Trial of StemRegenin-1 Expanded Umbilical Cord Blood Hematopoietic Stem Cells Supports Testing as a Stand-Alone Graft

John E. Wagner, Jr.,[1,2,*] Claudio G. Brunstein,[1] Anthony E. Boitano,[3] Todd E. DeFor,[1] David McKenna,[2] Darin Sumstad,[2] Bruce R. Blazar,[1] Jakub Tolar,[1] Chap Le,[1] Julie Jones,[4] Michael P. Cooke,[3] and Conrad C. Bleul[4]
[1]Blood and Marrow Transplant Program, University of Minnesota, Minneapolis, MN 55455, USA
[2]Molecular and Cellular Therapeutics Facility, University of Minnesota, Minneapolis, MN 55108, USA
[3]Genomics Institute of the Novartis Research Foundation, San Diego, CA 92121, USA
[4]Novartis Institutes for BioMedical Research, Basel 4000, Switzerland
*Correspondence: wagne002@umn.edu
http://dx.doi.org/10.1016/j.stem.2015.10.004

SUMMARY

Clinical application of umbilical cord blood (UCB) as a source of hematopoietic stem cells for transplantation is limited by low CD34+ cell dose, increased risk of graft failure, and slow hematopoietic recovery. While the cell dose limitation is partially mitigated by using two UCB units, larger-dosed single units would be preferable. We have evaluated the feasibility and safety of StemRegenin-1 (SR-1), an aryl hydrocarbon receptor antagonist that expands CD34+ cells, by placing one of the two units in expansion culture. SR-1 produced a 330-fold increase in CD34+ cells and led to engraftment in 17/17 patients at a median of 15 days for neutrophils and 49 days for platelets, significantly faster than in patients treated with unmanipulated UCB. Taken together, the marked expansion, absence of graft failure, and enhanced hematopoietic recovery support testing of SR-1 expansion as a stand-alone graft and suggest it may ameliorate a limitation of UCB transplant.

INTRODUCTION

Unrelated donor umbilical cord blood (UCB) is a major source of hematopoietic stem cells (HSCs) for transplantation with more than an estimated 30,000 recipients to date (Ballen et al., 2013). While UCB has the advantages of rapid availability, absent donor attrition, and relatively less human leukocyte antigen (HLA) restriction compared to HSCs from adult volunteer donors (Smith and Wagner, 2009; Cheuk, 2013), the use of UCB is substantially limited by the low finite number of HSCs and progenitor cells that can be collected from a placenta, resulting in prolonged periods of neutropenia, thrombocytopenia, and suboptimal engraftment and impeding its widespread application (Ballen et al., 2013; Smith and Wagner, 2009; Cheuk, 2013; Rubinstein et al., 1998; Scaradavou, 2010). Furthermore, studies have demonstrated an association between infused CD34+ and colony-forming unit-granulocyte macrophage (CFU-GM) cell doses and pace of hematopoietic recovery, non-relapse mortality, and survival (Wagner et al., 2002; Migliaccio et al., 2000; Page et al., 2011). As a result, there is considerable interest in finding ways to increase the absolute number of hematopoietic cells in an UCB graft, such as with of ex vivo expansion culture before transplantation.

StemRegenin-1 (SR-1) was first identified in an unbiased screen for compounds that promoted expansion of CD34+ hematopoietic progenitors (Boitano et al., 2010). SR-1 expanded HSCs retained multi-lineage potential and significantly augmented early and late engraftment of human cells in immune-deficient murine recipients. SR-1's effect on CD34+ cell expansion is mediated through direct binding and inhibition of the aryl hydrocarbon receptor, which normally promotes HSC differentiation during cytokine-driven expansion culture. Preclinical data demonstrate that SR-1 in the presence of stem cell factor (SCF), Fms-related tyrosine kinase 3 ligand (FLT-3L), thrombopoietin (TPO), and interleukin-6 (IL-6) leads to greater numbers of CD34+ cells when compared to previously reported expansion methods being evaluated in clinical trials (de Lima et al., 2008, 2012; Delaney et al., 2010; Horwitz et al., 2014). To explore the clinical utility of SR-1 mediated expansion, a current Good Manufacturing Practice (cGMP) expansion protocol was developed using SR-1 to expand UCB CD34+ cells and manufacture the product, referred to as HSC835, to speed hematopoietic recovery after transplantation.

The double UCB transplant platform, i.e., the infusion of two partially HLA-matched UCB units, was pioneered at the University of Minnesota (Barker et al., 2001, 2005; Brunstein et al., 2007) as a clinical strategy for evaluating the safety and effectiveness of graft manipulations, including the testing of expanded CD34+ cells. With this approach, one unit can be left unmanipulated while the other is placed in expansion culture, offering two significant advantages: (1) enhanced safety by incorporating an unmanipulated unit as a backup should the expansion culture fail or interfere with engraftment and (2) a means of tracking the relative contributions of the expanded and unmanipulated UCB units to hematopoietic recovery over time based on the inherent genetic differences between donors. Therefore, as in other recent trials evaluating hematopoietic cell expansion (de Lima et al., 2008, 2012; Delaney et al., 2010; Horwitz et al., 2014), we used

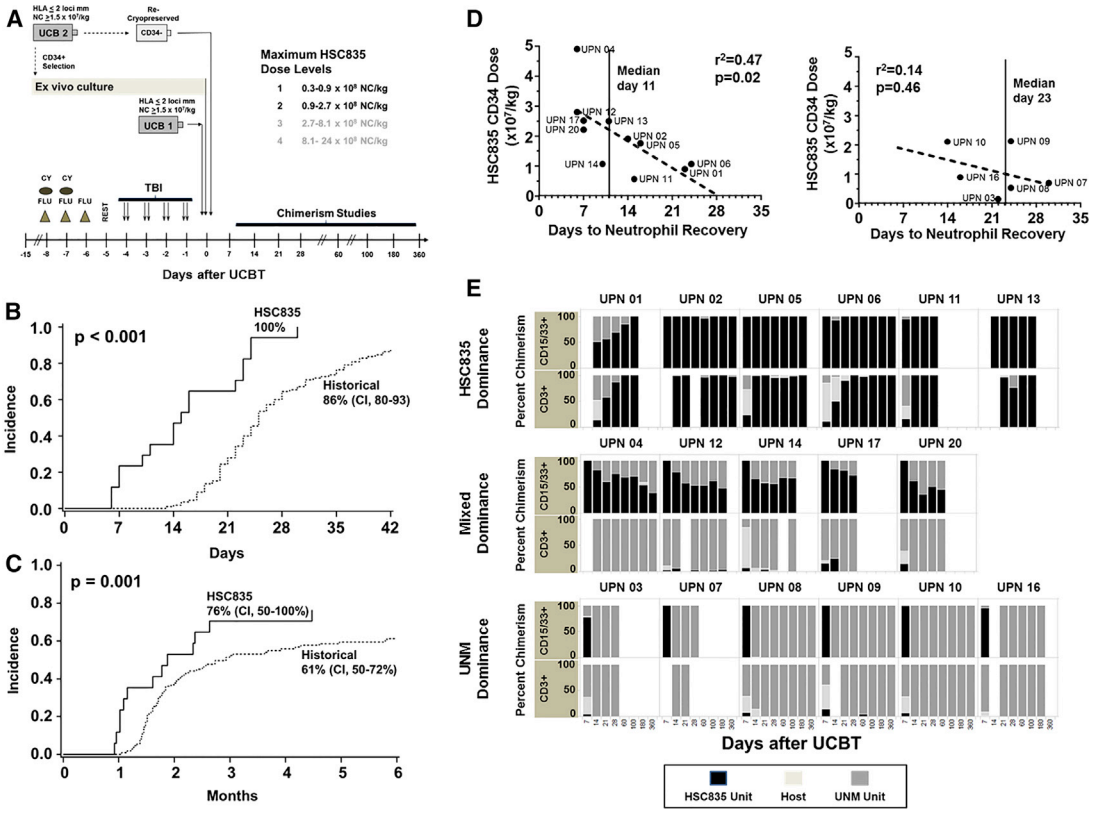


Figure 1. Trial Design and Engraftment Outcome

(A) Schematic of the treatment plan. On day −15 before transplant, CD34+ cells were enriched from the lower cell-dosed UCB unit (UCB 2) and placed in expansion culture for 15 days, including 50 ng/ml each of SCF, FLT-3L, TPO, and IL-6, and 750nM SR-1. CD34− cells were collected and recryopreserved. Between days −8 and day −1, the patient was conditioned with cyclophosphamide (CY) 60 mg/kg/day (days −8 and −7), fludarabine (FLU) 25 mg/m^2/day (days −8, −7, and −6), and total body irradiation (TBI) 165 cGy twice daily (days −4, −3, −2, and −1). On the day of transplant (day 0), the unmanipulated higher cell-dosed unit (unit 1) was infused first, with HSC835 infused 4 hr later. CD34− cells were infused 4–24 hr after HSC835. Dose levels are shown; however, only dose levels 1 and 2 were evaluated in this trial.

(B) Incidence of neutrophil recovery for patients transplanted with HSC835 (n = 17) compared to that in the historical cohort (n = 111). Neutrophil recovery was defined as ANC $\geq$ 0.5 × 10^9/l for 3 consecutive days.

(C) Incidence of platelet recovery for patients transplanted with HSC835 (n = 17) compared to that in the historical cohort (n = 111). Platelet recovery was defined as platelet count $\geq$ 0.5 × 10^{10}/l for 7 consecutive days without transfusion.

(D) Correlation between HSC835 CD34+ cell dose and time to neutrophil recovery. For those with HSC835 predominance in the CD15/CD33 myeloid cell population, neutrophil recovery is rapid, with a strong correlation between HSC835 CD34+ cell dose and time to recovery (left panel). For those with predominance of the unmanipulated unit, recovery is slower, with no correlation between HSC835 CD34+ cell dose and time to recovery (right panel).

(E) Patterns of chimerism in the CD3+ T cell and CD15/CD33+ myeloid cell populations for each patient, identified by a UPN. In the upper panel, chimerism in myeloid (CD15/CD33) and T cell (CD3) lineages is principally derived from the HSC835 unit. In the middle panel, chimerism in the myeloid lineage is mixed, with contributions from both HSC835 and the unmanipulated unit, and lymphoid lineage is almost exclusively derived from the unmanipulated unit. In the lower panel, chimerism in both lineages is nearly completely derived from the unmanipulated units, with brief HSC835 predominance at day 7.

the double UCB platform to explore the safety and efficacy of HSC835.

RESULTS

Patient and Donor Graft Characteristics

In the study, 20 patients were enrolled and 17 completed the prescribed treatment plan receiving HSC835 after myeloablative conditioning in the double UCB transplant setting (Figure 1A). Demographics and graft characteristics for the recipients of HSC835 are shown (Table 1), along with the characteristics of a comparison historical control cohort selected on the basis of disease type and similarities in treatment plan (i.e., conditioning and graft-versus-host disease [GVHD] prophylaxis), to assess the safety profile of HSC835 in terms of hematopoietic recovery and engraftment. All patients had leukemia or advanced myelodysplastic syndrome and similar performance status. Donor-recipient HLA match and blood group A, B, and O (ABO) compatibility were similar as well. By study design, the most desirable of the two units based on cell dose and HLA match was left unmanipulated (unit 1), while the second best unit with a lower cell dose (unit 2) was selected for ex vivo expansion culture. As expected, HSC835 had significantly greater numbers of CD34 cells compared to unmanipulated units (Table 2), with a median of 17.5 × 10^6 CD34 (range, 1.4–48.3) per kilogram actual body weight for HSC835 units in contrast to the median number of

Table 1. Characteristics of Patients

Demographics	Expansion Cohort (n = 17)		Historical Conventional Cohort (n = 111)		p value
Median recipient age (range)	29.9 years (12–54)		25.6 years (11–51)		0.07
Median recipient weight (range)	87.1 kg (42–130)		69.6 kg (32–149)		0.09
Diagnosis	–		–		0.01
Acute Myelocytic Leukemia					
First CR	4 (23.5%)		33 (29.7%)		
Second CR	–		24 (21.6%)		
Third CR	–		4 (3.6%)		
Acute Lymphocytic Leukemia					
First CR	10 (58.8%)		30 (27.0%)		
Second CR	–		14 (12.6%)		
Third CR	1 (5.9%)		2 (1.8%)		
Myelodysplastic syndrome	2 (11.8%)		4 (3.6%)		
CMV Serostatus					
Recipient positive	9 (52.9%)		61 (55.0%)		0.88
Recipient negative	8 (47.1%)		50 (45.0%)		
HLA Match with Recipient (Maximum Disparity)	Unit 1[a]	Unit 2	Unit 1[a]	Unit 2	
6/6	3 (17.7%)	1 (5.9%)	9 (8.1%)	12 (10.8%)	0.27
5/6	9 (52.9%)	9 (52.9%)	34 (30.6%)	52 (46.9%)	
4/6	5 (29.4%)	7 (41.2%)	68 (61.3%)	47 (42.3%)	
ABO Match with Recipient	Unit 1[a]	Unit 2	Unit 1[a]	Unit 2	
Match	5 (29.4%)	7 (41.2%)	36 (32.4%)	23 (20.7%)	0.07
Major mismatch	12 (70.6%)	10 (58.8%)	53 (47.8%)	53 (47.8%)	
Minor mismatch	0	0	22 (19.8%)	35 (31.5%)	
Performance Score					
100	8 (47.1%)		54 (48.6%)		0.59
90	8 (47.1%)		55 (49.5%)		
80	1 (5.9%)		2 (1.8%)		
Conditioning and GVHD Prophylaxis					
CY 120 mg/kg, FLU 75 mg/m2, TBI 1,320 cGy	100%		100%		NS
CYclosporin A and mycophenolate mofetil	100%		100%		NS

CR, complete remission; CY, cyclophosphamide; FLU, fludarabine; TBI, total body irradiation (total doses shown).
[a]Unit 1 is the larger of the two units.

0.2×10^6 CD34+ cells/kg present in unmanipulated UCB units. In contrast, the CD3 cell dose was significantly less in recipients of HSC835, because it was derived from the smaller unit 2 and had resultant nonspecific losses with re-cryopreservation of the CD34− fraction, which was collected 15 days before transplantation.

SR-1 Expansion Culture Reliably Provides Greater than 200-fold Expansion of CD34+ Cells

Cell numbers at each step of the HSC835 manufacturing process (CD34 selection and expansion culture) are shown for each patient (Table 3). After CD34+ cell selection, a median of 4.4×10^6 total CD34+ cells (range, 2.1–14.3) were placed in expansion culture. After 15 days, a median of $1,440 \times 10^6$ total CD34+ cells (range, 140–6,361) were recovered. Based on these results, SR-1 expansion cultures yielded a median of a 330-fold (range, 67–848) increase in the number of CD34+ cells and an 854-fold (range, 168–2,121) increase in numbers of total nucleated cells (TNC). In three patients, each identified by a unique patient number (UPN; UPN04, UPN17, and UPN20), the final HSC835 product exceeded the maximum allowable cell dose permitted, resulting in the infusion of only a proportion of the cells available (see Dose Capping). Importantly, the fold expansion of CD34+ cells was independent of the starting CD34 number. In all but one case (UPN03), CD34+ cell expansion exceeded >200-fold. For UPN03, expansion kinetics differed from all other cultures in that there was no evidence of expansion in nucleated cells after the first 3 days of culture.

Eighteen HSC835 products met lot release (see Unit Processing and Table S1). Two products (for UPN15 and UPN19) failed lot release due to a positive bacterial culture. In addition, one product (UPN13) that met lot release had a positive surveillance culture reported 18 hr after infusion. While this patient's blood cultures remained negative after transplant, transient

Table 2. Graft Characteristics

Cells Infused Median (Range)	HSC835 Cohort (n = 17)				Historical Conventional Cohort (n = 111)			
	Unit 1	Unit 2 Pre-expansion	Unit 2 Post-expansion	Combined[a]	Unit 1[b]	Unit 2	Combined[a]	p value
Nucleated cells × 10^8/kg	0.2 (0.2–0.5)	0.2 (0.1–0.6)	0.5 (0.1–1.2)	0.7 (0.3–1.7)	0.2 (0.1–0.5)	0.2 (0.1–0.5)	0.4 (0.2–1.0)	<0.001
CD34 cells × 10^6/kg	0.4 (0.2–0.9)	0.2 (0.0–1.0)	17.5 (1.4–48.3)	18.2 (2.3–48.5)	0.2 (0.0–1.1)	0.3 (0.1–1.0)	0.5 (0.1–2.1)	<0.001
CD3 cells × 10^6/kg[c]	8.4 (4.6–28.8)	ND	2.9[d] (0.4–5)	12.3 (5–33)	7.0 (2–26)	9.0 (3–21)	16 (5–47)	0.28
CFU-GM colonies × 10^4/kg	3.3 (1.2–7.4)	ND	389.1 (40.7–1,335.8)	394.5 (47.8–1,338.4)	1.9 (0.1–8.9)	2.0 (0.0–14.0)	3.9 (0.1–22.9)	<0.001

ND, not determined.

[a]Combined cell dose were compared by general Wilcoxon test.

[b]Unit 1 is the larger of the two units.

[c]CD3+ cells were derived solely from the cryopreserved CD34 depleted fraction.

[d]p value < 0.001, comparing the HSC835 CD3 cell dose to that in the unmanipulated unit.

hypotension occurred 4 hr after infusion, which was the only observed infusional toxicity event. For the three positive cultures, the isolates differed (*Propionibacterium acnes* [UPN13], coagulase negative *Staphylococcus* [UPN15], and *Leifsonia aquatica* [UPN19]). Process improvements in product manufacture, specifically batch production of expansion media and reduced in-process testing, subsequently resulted in 13 of 13 validation study and clinical products meeting lot release.

HSC835 Promotes Rapid Hematopoietic Recovery

For the 17 recipients of HSC835, neutrophil recovery was achieved in all patients at a median of 15 days (6–30 days), while 86% patients in the control group recovered at a median of 24 days (Figure 1B). Like neutrophil recovery, platelets also recovered more rapidly at a median of 49 days (28–136 days) versus 89 days (p = 0.001), respectively. Six months after transplantation, 76% (CI, 50%–100%) of HSC835 recipients achieved platelet transfusion independence compared to 61% (CI, 50%–72%) of historical controls (Figure 1C).

After double UCB transplant, we and others have previously shown that one unit typically predominates and the second unit is lost (Barker et al., 2001, 2005; Brunstein et al., 2007). In this study, the origin of hematopoietic recovery (unit 1 versus unit 2 versus host) was determined at fixed time points by discriminating short tandem repeat loci in sorted CD15+/CD33+ myeloid cells, the most prevalent population in the peripheral blood after transplant (Figure 1D). Hematopoiesis was principally derived from the expanded unit (HSC835) in 11 patients and the unmanipulated unit in 6 patients. In those engrafting with HSC835, time to neutrophil recovery was more rapid at a median 11 days (range, 6–23 range) and correlated with CD34+ cell dose (Figure 1D, left panel). In contrast, no correlation was observed in the 6 patients engrafting with the unmanipulated unit, and speed of recovery was significantly slower (median, 23 days; range, 14–30 range). For those engrafting with HSC835, myeloid engraftment was durable (median follow-up, 272 days; range, 35–688 range) (Figure 1E).

A detailed analysis of chimerism, including an evaluation of sorted CD3 cells in the peripheral blood, revealed three patterns: (1) in six patients, the CD15/CD33 (myeloid) and CD3 (T cell) populations are entirely derived from HSC835 (Figure 1E, upper panel); (2) in six patients, the CD15/CD33 and CD3 populations are entirely derived from the unmanipulated unit (lower panel); and (3) in five patients, a unique chimerism pattern was observed with the CD15/CD33 population predominantly derived from HSC835 and the CD3 population almost exclusively derived from the unmanipulated unit (middle panel). These five patients (UPN04, UPN12, UPN14, UPN17, and UPN20) with myeloid chimerism from both HSC835 and the unmanipulated unit had particularly rapid neutrophil recovery (median, 7 days; range, 6–10 days).

Unit Predominance Is Associated with Graft-versus-Graft Immune Reactivity

In all patients, HSC835 myeloid chimerism was near complete on day 7. However, it was not sustained in six patients. As shown in Figure 2A, a more detailed analysis of the HSC835 product demonstrated expansion of the CD34+CD133+CD90+ in all products, the population enriched for the HSCs (Radtke et al., 2015; Wong et al., 2013). Since there was an even greater expansion of committed progenitors (CD34+CD133+CD90− and CD34+CD133−CD90−), these data suggest that the expansion culture did not have a deleterious effect on the engraftment potential of HSC835 even in the six patients ultimately engrafting with the unmanipulated unit. Based on the report by Gutman et al. (2010) documenting a specific CD8+ T cell response of the predominating unit against the non-engrafting unit, we examined the peripheral blood of each patient early after transplant for evidence of alloreactive T cells directed against the non-engrafting unit. Of seven patients in whom sufficient numbers of CD8+ T cells could be recovered, five patients had interferon-gamma (IFN-γ)-producing T cells in the peripheral blood derived from the predominating unit in response to the non-engrafting unit (Figure 2B). Specifically, UPN02 and UPN05 showed reactivity against the unmanipulated unit and engrafted with HSC835, and UPN03, UPN09, and UPN10 showed reactivity against HSC835 and engrafted with the unmanipulated unit. In the two remaining patients, one had no reactivity to either HSC835 or the unmanipulated unit and engrafted with both HSC835 and the unmanipulated unit (UPN04), and one had borderline reactivity (UPN08) to HSC835 and engrafted with the unmanipulated unit.

Having previously shown an association between the unit's CD3+ cell dose and not nucleated or the CD34+ cell dose and unit predominance (Brunstein et al., 2007; Radtke et al.,

Table 3. Selection and Expansion Profiles

		Cryopreserved		After Thawing			After CD34 Selection		HSC835 (After Expansion)				CD34 Depleted (Infused)		
UPN	Patient Weight (kg)	Total NC (x 10^8)	Total CD34+ (x 10^6)	Total NC (x 10^8)	Total CD34+ (x 10^6)	CD34 Recovery (%)[a]	Total NC (x 10^6)	Total CD34+ (x 10^6)	Total NC (x 10^8)	Total CD34+ (x 10^6)	Fold CD34 Expansion	Fold NC Expansion	Total NC (x 10^8)	Total CD34+ (x 10^6)	Total CD3+ (x 10^6)
01	86.2	14.2	4.5	11.6	6.6	146.9	2.8	2.6	23.9	965.8	371	854	4.8	1.5	227.7
02	119.9	31.2	9.6	24.5	14.7	153.3	11.5	10.5	49.6	2,496.9	238	431	14.1	6.2	592.7
03	64.5	13.4	5.2	12.0	7.4	142.4	2.2	2.1	3.7	140.2	67	168	4.0	0.2	185.7
04[b]	110.9	20.6	14.3	17.6	31.7	221.9	15.6	14.3	118.4	6,361.9	445	759	7.9	3.1	308.9
05	102.5	27.5	12.4	21.6	15.1	122.0	9.2	8.2	90.4	3,066.5	374	982	13.1	5.8	393.0
06	89	14.2	8.7	9.6	8.5	97.9	5.2	4.9	41.7	1,191.5	243	802	2.5	0.2	39.4
07	131.2	22.9	11.5	22.1	7.1	61.7	5.2	4.3	30.4	1,123.0	261	585	9.5	0.8	85.1
08	90.7	16.6	3.3	13.5	9.5	284.0	2.8	2.3	22.3	461.8	201	796	5.3	2.4	237.6
09	63.1	11.6	7.5	10.4	13.0	171.9	7.0	6.0	58.4	1,483.0	247	834	5.0	0.8	246.1
10	68.9	21.1	12.7	17.7	17.2	135.8	5.8	2.9	97.9	2,460.3	848	1,688	8.8	1.3	210.8
11	84.3	14.4	5.8	11.9	6.4	112.0	2.9	2.6	38.7	746.1	287	1,334	7.2	2.7	229.2
12	63.1	18.1	9.0	15.7	13.2	146.9	4.6	4.2	97.6	2,557.8	609	2,121	5.3	0.5	257.3
13	52.4	10.1	7.7	8.4	10.6	137.1	4.7	4.5	95.3	2,175.9	484	2,028	4.7	0.7	102.4
14	113.5	19.0	5.4	18.1	12.5	231.8	4.5	3.7	50.0	1,440.3	389	1,111	5.9	0.5	199.0
15	48.0	22.5	9.2	19.1	17.4	188.9	6.4	6.0	NA	NA	NA	NA	NA	NA	NA
16	105.9	20.8	6.4	17.5	13.6	211.4	4.1	3.4	32.4	1,183.7	348	790	8.7	2.5	339.8
17[b]	40.5	29.7	21.4	22.4	41.2	192.7	12.2	9.7	115.0	3,196.6	330	943	6.8	1.9	190.0
18	44.9	17.7	7.8	14.3	13.8	177.3	4.0	3.6	43.2	1,212.7	337	1,080	NA	NA	NA
19	113.2	23.3	5.8	20.0	17.6	304.3	6.1	5.9	57.5	1,241.1	210	943	NA	NA	NA
20[b]	49	19.1	19.9	16.6	34.1	171.6	8.0	7.8	43.3	1,677.5	215	541	7.8	3.4	195.3
Median	86.2	19.1	8.3	17.1	13.4	162.5	5.2	4.4	49.6	1,440.3	330	854	6.8	1.5	227.7

Numbers of cells infused do not match the total numbers manufactured, because samples were sent for lot release or quality control and research assays. HSC835, expanded unit; CD34 depleted, residual cells after CD34 selection (these were recryopreserved and infused 4–24 hr after HSC835 infusion); NC, nucleated cell count; NA, not available.

[a]Methodology in the University of Minnesota Medical Center Clinical Laboratory differs from that in the Cord Blood Banks, resulting in a uniformly higher percentage of CD34 than previously reported (Flores et al., 2009).

[b]Cell expansion exceeded the dose allowed; patients received the maximum allowed at dose level 1.

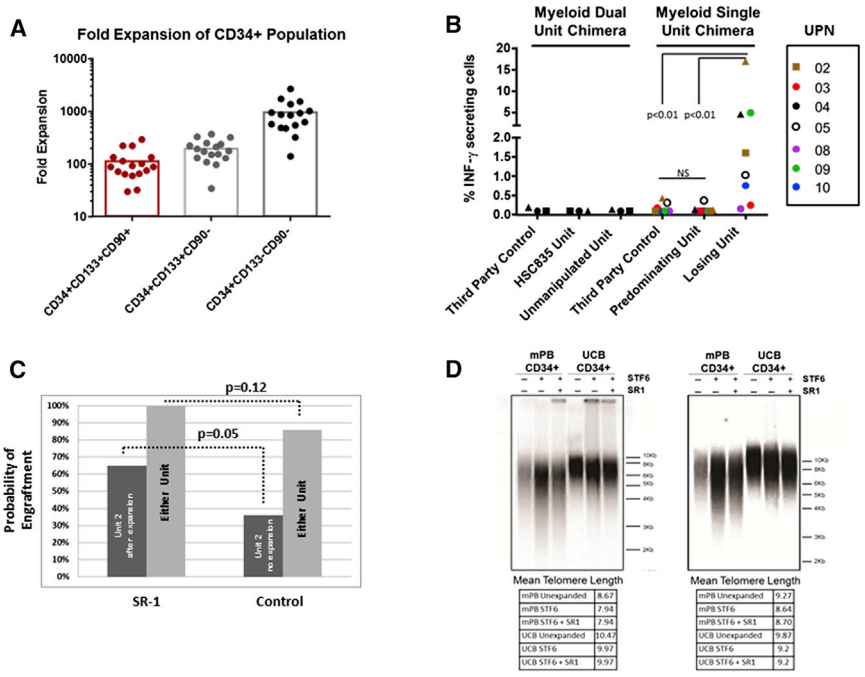


Figure 2. Analysis of Risk Factors Potentially Influencing HSC835 Reconstitution

(A) Fold expansion of CD34+ subpopulations based on co-expression of CD133+ and CD90+ in recipients of HSC835.

(B) Graft-versus-graft immune reactivity. Proportion of IFN-γ-secreting T cells obtained from the peripheral blood of seven patients between day 14 and day 56 after UCB transplant in response to Epstein-Barr virus-transformed HSC835, unmanipulated unit, and third-party donor cells. The box shows the UPN for each patient with a sufficient number of peripheral blood mononuclear cells for evaluation, which were obtained on day 14 (triangles), day 28 (circles), or day 56 (squares) after transplant, with colors indicating individual patients.

(C) Probability of engraftment in recipients of double UCB transplant with and without SR-1 expanded hematopoietic cells. Engraftment of the lower cell-dosed unit 2 is shown with and without SR-1 expansion (black bar). Overall engraftment by day 42 after double UCB transplant (regardless of which unit predominates) is also shown (gray bar).

(D) Panels show the telomere length of mobilized peripheral blood (mPB) from normal donors and UCB CD34+ cells, which were evaluated before and following culture in SCF, FLT-3L, TPO, and IL-6 (SFT6) with and without SR-1. Each plot is an individual experiment from two independent donors. Tables beneath each plot indicate the telomere length as measured in kilobase pairs.

2015; Wong et al., 2013; Gutman et al., 2010; Ramirez et al., 2012), corroborated by preclinical studies demonstrating the importance of T cells on unit predominance in non-obese diabetic-severe combined immunodeficiency recipient engraftment (Lund et al., 2015; Yahata et al., 2004), we hypothesized that the frequency of unit 2 predominance would be similar regardless of expansion culture. Therefore, we compared the frequency of unit 2 predominance after expansion culture (i.e., HSC835 and its associated CD34− fraction) with unit 2 without expansion culture in the historical cohort. As shown (Figure 2C), unit 2 was more likely to expand after expansion culture ($p = 0.05$), which is particularly remarkable considering the additional losses of CD3+ T cells with re-cryopreservation of the CD34− fraction. While no patient in the study cohort experienced graft failure, there were insufficient numbers of patients to document a reduction in graft failure after the transplantation of two unmanipulated units ($p = 0.12$). Together, these data suggest that an immune response between units accounted for unit predominance and expansion culture did not interfere with the unit's repopulation potential.

Effect of HSC835 on Other Transplant Outcomes

The primary endpoint of the clinical trial was to determine the safety of HSC835, with secondary endpoints on the various transplant outcomes (Table 4). Other than transient hypotension with an occult bacterial contamination of the HSC835 product in one patient (UPN13), no infusional toxicities were noted within the first 24 hr after transplant and no other adverse effects were observed that could be attributed to the HSC835 infusion. As shown in Figure S1, the adverse event profiles between day 0 and day 30 were comparable to those commonly observed after UCB transplant. In addition, no patient had late graft failure within the follow-up period. Of patients engrafting with HSC835, the longest follow-up was 687+ days (UPN05). On this point, we also examined telomere length before and after UCB expansion culture as a potential risk factor for early HSC senescence. As shown in Figure 2D, expansion culture with SR-1 resulted in an approximately 500 base pair reduction in mean telomere length. However, the telomere length of expanded UCB CD34+ cells compared favorably to Neupogen-mobilized adult peripheral blood CD34+ cells without expansion culture, which is the most commonly used HSC source for transplantation in adults.

In terms of other transplant outcomes, risk of grade II–IV and grade III–IV acute GVHD, transplant-related mortality, and overall survival was similar compared to the historical cohort (Figures 3A–3C, respectively). The length of hospitalization was significantly reduced at a median of 30 days compared to 46 days in the control population ($p < 0.001$).

Lastly, exploratory assessments of T and B cell recovery were performed. As shown (Figure 3D), the absolute number of CD3+, CD4+, and CD4+CD45RA+CCR7+ cells is lower at day 28 in those patients for whom the T cells emanate from the CD34− fraction associated with HSC835 than in other patients for whom the T cells are derived from the unmanipulated product. Because early T cell recovery reflects homeostatic expansion of the mature T cells infused (Politikos and Boussiotis, 2014; Krenger et al., 2011), the initial lag may reflect the lower number of T cells in the recryopreserved CD34− fraction associated with HSC835. Because opportunistic infection and relapse have been associated with poor immune recovery, cytomegalovirus (CMV) interstitial pneumonitis was the primary cause of death in one

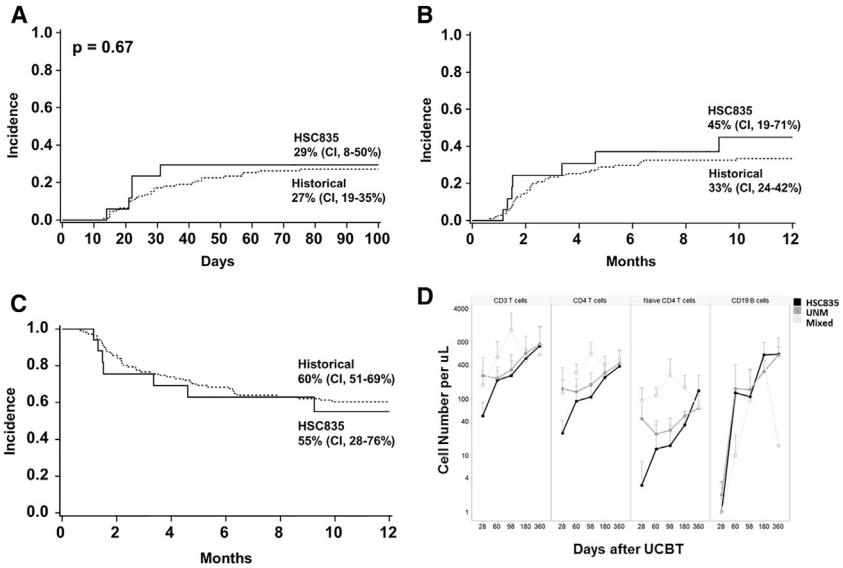


Figure 3. Effects of HSC835 on Clinical Transplant Outcomes and Immune Recovery

(A) Incidence of grade III–IV acute GVHD in recipients of HSC835 (n = 17) relative to the historical control cohort (n = 111).

(B) Incidence of transplant-related mortality, defined as mortality due to any cause other than relapse.

(C) Probability of survival, defined as death from any cause, including relapse.

(D) Patterns of immune recovery. Mean cell numbers (± SE) per microliter of peripheral blood are shown for CD3, CD4, CD4/CD45RA/CCR7, and CD19 lymphocyte subsets on days 28, 60, 98, 180, and 360 after UCB transplant. Patients are grouped on the basis of which units contributed to hematopoietic recovery, specifically, HSC835 only (n = 5), unmanipulated (UNM) unit only (n = 4), or both HSC835 and UNM units (mixed, n = 4).

patient and relapse was not observed within the follow-up period. Other primary causes of death were acute GVHD (n = 4), chronic GVHD (n = 1), and idiopathic (n = 1) and diffuse alveolar hemorrhage (n = 2).

DISCUSSION

Low stem cell dose resulting in prolongation or failure of lympho-hematopoietic recovery after UCB transplant has markedly limited the success HSC source. Today, however, there are multiple promising strategies for enhancing the pace of hematopoietic recovery after UCB transplant. Notch-ligand, adherent mesenchymal stromal cell, and nicotinamide-based expansion cultures are each associated with rapid neutrophil recovery at a median of 16, 15, and 13 days, respectively (Delaney et al., 2010; de Lima et al., 2012; Horwitz et al., 2014). However, neutrophil recovery from the expanded product is transient after Notch-ligand and mesenchymal stromal cell culture, with long-term engraftment emanating from the unmanipulated unit in nearly all instances (Delaney et al., 2010; de Lima et al., 2012). In contrast, nicotinamide-cultured cells, like SR-1 in this study, not only promote rapid recovery but also lead to sustained engraftment in a substantial proportion of patients (Horwitz et al., 2014). While mechanism of action for nicotinamide remains to be elucidated, emerging data suggest that it may also enhance homing to the marrow microenvironment. Other methods to improve homing, as with the inhibition of dipeptidylpeptidase 4/CD26, treatment of cells with 16,16-dimethyl prostaglandin E2, and fucosylation, are also promising, with neutrophil recovery reported at a median of 17.5, 21, and 14 days, respectively, in initial pilot trials (Farag et al., 2013; Cutler et al., 2013; Popat et al., 2015).

In this study, several important findings emerge: (1) SR-1 in the presence of specific cytokines promotes remarkable CD34+ cell expansion, greater than that observed by all other methods previously tested in clinical trials (de Lima et al., 2008, 2012; Delaney et al., 2010; Horwitz et al., 2014), albeit in the progenitor compartment predominantly; (2) infusion of HSC835 is well tolerated; and (3) the pace of neutrophil and platelet recovery is significantly accelerated, correlating with HSC835 CD34 cell dose. Despite potential adverse effects with such marked expansion, clonal cytogenetic abnormalities or excess telomere shortening was not observed in any expanded product and engraftment has been durable, further supporting its safety profile.

In patients with HSC835 predominance, the pace of hematopoietic recovery was remarkably rapid, especially in those with a CD34 cell dose greater than ten million/kg. In addition, HSC835 contributed to sustained hematopoiesis in 11 of 17 patients, which contrasts with UCB hematopoietic progenitors expanded in the presence of Notch ligand (Delaney et al., 2010) or adherent mesenchymal progenitor cells (de Lima et al., 2012), which contribute to hematopoiesis only transiently. While HSC exhaustion cannot be ruled out, it is possible that the transient recovery observed with these methods was due to the absence of CD3+ cells, leaving the expanded unit at an immunological disadvantage. Like us, Horwitz et al. (2014) infused the recryopreserved fraction containing T cells and observed sustained engraftment. Graft-versus-graft immune responses play a role in unit predominance after double UCB transplant (Gutman et al., 2010; Ramirez et al., 2012). We have previously shown that the unit with the larger CD3+ cell dose is more likely to predominate (odds ratio, 4.4 [95% CI 1.8–10.6]; $p < 0.01$) (20). This pattern, however, did not hold true for HSC835. After expansion, the HSC835 product out-competed the unmanipulated unit in 11 of 17 transplants despite lower CD3 cell doses in all cases. This suggests that ''megadoses'' of CD34+ cells may overcome the engraftment barrier, as suggested in preclinical models (Reisner et al., 2003).

While transient engraftment has had a demonstrable clinical effect with some expansion methodologies (Delaney et al., 2010; de Lima et al., 2012), the transient burst of myeloid cells derived from HSC835 detected on day 7 had no obvious impact on shortening the period of neutropenia in those that ultimately engrafted with the unmanipulated unit. In about one-third of patients, however, mixed myeloid chimerism derived from both units was associated with very rapid neutrophil recovery.

Table 4. Graft Characteristics and Transplant Outcomes

UPN (Dose Level)[a]	Infused TNC (10^7/kg)[b]		Infused CD34 (10^6/kg)[b]		Infused CFU-GM (10^4/kg)[b]		Infused CD3 (10^6/kg)[b]		Days to ANC ≥ 5×10^8/l	Predominant Unit[c]		Days to PLT ≥ 20×10^9/l	Max Grade Acute GVHD	Survival (Days)
	UNM	HSC835	UNM	HSC835	UNM	HSC835	UNM	HSC835		CD3	CD15			
01 (1)	1.8	2.8	0.3	8.9	1.9	121.7	7.2	2.6	23	HSC835	HSC835	80	3	140
02 (1)	2.0	5.0	0.4	19.1	3.3	639.4	7.3	5.0	14	HSC835	HSC835	136	3	442
03 (1)	2.4	1.0	0.9	1.4	7.0	40.7	6.6	2.9	22	UNM	UNM	NA	0	40
04 (1)	1.8	9.7[d]	0.2	48.3	2.4	1,325.1	8.4	2.8	6	UNM	HSC835	54	3	688
05 (1)	2.8	6.4	0.7	17.5	3.6	523.2	9.1	3.8	16	HSC835	HSC835	72	2	687+
06 (1)	2.1	4.0	0.5	10.7	2.1	275.0	6.6	1.1	23	HSC835	HSC835	57	0	623+
07 (1)	1.5	2.6	0.2	7.0	2.0	231.0	4.6	2.3	30	UNM	UNM	NA	0	45
08 (1)	3.5	3.1	0.3	5.3	5.4	389.1	10.5	2.6	24	UNM	UNM	35	0	545+
09 (1)	2.0	9.1	0.3	21.2	2.6	1,335.8	8.9	4.0	24	UNM	UNM	49	0	539+
10 (1)	2.9	9.6	0.6	21.0	7.4	1,005.2	12.5	3.2	14	UNM	UNM	33	3	391+
11 (1)	3.4	3.8	0.7	5.7	5.0	255.1	7.3	2.8	15	HSC835	HSC835	NA	0	46
12 (2)	5.2	12.1	0.6	29.6	4.5	144.8	28.8	4.3	6	UNM	HSC835	31	2	281
13 (2)	3.7	12.3	0.5	26.1	2.6	662.2	13.7	2.2	11	HSC835	HSC835	29	2	272+
14 (1)	2.0	4.2	0.3	10.6	1.2	292.1	7.1	1.9	10	UNM	HSC835	28	3	102
16 (1)	1.8	3.3	0.3	8.9	3.0	148.4	6.0	3.2	16	UNM	UNM	71	2	238+
17 (1)	2.5	10.7[d]	0.2	25.2	4.8	780.8	14.6	4.7	7	UNM	HSC835	NA	2	35
20 (1)	4.2	10.6[d]	0.4	34.9	6.0	637.5	13.9	4.4	7	UNM	HSC835	31	0	43+
Median	2.4	5.0	0.4	17.5	3.3	389.1	8.4	2.9	15			49		

HSC835, expanded unit; UNM, unmanipulated unit; PLT, platelet count; NA, not achieved.

[a]Patients were eligible for dose level 2 if no DLTs were observed at the prior dose level in at least three patients and the expansion culture yielded sufficient cells. Pediatric recipients were evaluated at each dose level separate from adults.

[b]HSC835 graft is the sum of cell populations from HSC835 and the CD34 depleted fraction.

[c]Predominant unit on day 60 (or last follow-up) after UCB transplant for CD3 (T) and CD15/CD33+ (myeloid) cell subsets.

[d]Cell expansion exceeded the dose allowed at the prescribed dose level.

Chimerism from two units (beyond 60 days) rarely occurs after double UCB transplant. Perhaps larger numbers of CD34+ cells and/or the reduction in the T cell dose influenced this outcome. For the entire cohort, pace of platelet recovery was accelerated, although perhaps less dramatically than that observed for neutrophil recovery. However, chimerism from both units had no demonstrable impact on speed of platelet recovery, although small patient numbers prevent definitive conclusion. Perhaps the full benefit of a higher CD34+ cell dose on platelet recovery was diminished by the higher rates of GVHD and graft-versus-graft reactions generally observed after double UCB transplant (Wagner et al., 2014).

The data reported here suggest that graft-versus-graft interactions are likely responsible for the loss of HSC835 in the six patients, rather than a loss in engraftment potential. Like Gutman et al. (2010), we found IFN-γ-secreting T cells in patients directed against the losing unit in five of six patients in whom one unit predominated. Interestingly, in one patient with myeloid chimerism from both units and T cells derived from the unmanipulated unit, IFN-γ-secreting T cells could not be detected against either HSC835 or the unmanipulated unit, suggesting tolerance between unit 1 and unit 2.

The marked expansion of CD34+ cells with SR-1 suggests that the limitation of cell dose in recipients of UCB may have been ameliorated. In contrast to methods with a lower fold expansion of CD34+ cells, SR-1 obviates the need to target UCB units with higher CD34 doses and permits the utilization of UCB units previously considered to be unsuitable for general use due to low cell dose. Lowering the cell dose threshold for UCB transplant from 2.5×10^7 to 1×10^7 nucleated cells/kg, for example, not only markedly increases the size of the usable inventory, and therefore the chance of identifying a better HLA-matched unit, but also significantly reduces the cell count threshold for banking, which is currently more than one billion nucleated cells. This would be especially important for patients of ethnic and racial minorities in whom there is a tendency for lower cell counts in the units collected (Cairo et al., 2005). The availability of SR-1-based expansion could also have a profound effect on the economics of cord blood banking by improving collection efficiencies and the availability of suitable units for transplantation.

The primary aim of the trial was to document the safety profile of HSC835. While higher cell dose levels have yet to be evaluated in children, the toxicity profile is encouraging. Thus far, there has been a single infusional toxicity that was related to an occult bacterial contamination. After procedural changes in the manufacturing process, including process improvements in batch production of expansion media and reductions in in-process testing, all subsequent products have met lot release. Safety profile, rapidity of neutrophil and platelet recovery, frequency of HSC835 predominance, and reduction in the number of hospital days are sufficiently encouraging to justify further evaluation of HSC835 (in combination with its CD34− fraction) as a "stand-alone" graft. The infusion of a single expanded UCB unit will eliminate the confounding graft-versus-graft effects observed in the current study. While the co-infusion of an unmanipulated unit served as a safety "backup" should the expansion procedure have a deleterious effect on the manipulated unit's engraftment potential, double UCB transplant is associated with higher risks of GVHD and delayed platelet recovery. Therefore, future studies with a single expanded unit will more precisely define the impact of SR-1 expansion culture on the pace of hematopoietic recovery, engraftment, GVHD, transplant mortality, and survival, as well as the pace of immune recovery, resource utilization, health quality of life, and hospital days. The demonstration that SR-1 promotes marked expansion of hematopoietic stem and progenitor cells also suggests it may be a useful strategy for amplifying the number of genetically modified HSCs, a consideration for future studies (Naldini, 2011).

EXPERIMENTAL PROCEDURES

Study Design

This trial was an open-label, phase I/II, dose-escalation study with four predefined maximum cell dose levels (Figure 1A). The primary endpoint was safety, as measured by the absence of infusional toxicity or deleterious effects on hematopoietic recovery. Pre-specified secondary or exploratory endpoints included time to and incidence of engraftment, rate of neutrophil and platelet recovery, relative contribution of the two units to early and late hematopoietic recovery, and immune reconstitution. Other endpoints included GVHD, transplant-related mortality, and survival. Adverse event data (Figure S1) were reviewed by an independent Data and Safety Monitoring Board appointed by Novartis.

Consent from patients or guardians of minors was obtained in accordance with the Declaration of Helsinki. The work was approved by the Institutional Review Board of the University of Minnesota, and the clinical protocol was registered with ClinicalTrials.gov (NCT01474681) before study initiation.

Eligibility

To participate in this trial, patients had to be aged 10–55 years, inclusive, with high-risk hematological cancer as described previously (Wagner et al., 2002; Barker et al., 2005). Performance and organ function criteria included good general health, defined as a Karnofsky/Lansky score $\geq$ 80%; creatinine < 2.0 mg/dL; bilirubin, aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase more than three times the upper limit of normal; diffusion lung capacity for carbon monoxide corrected > 50% the upper limit of normal, and left ventricular ejection fraction > 45%. In addition, the patients could not have an active infection at time of transplantation (including active infection with *Aspergillus* or other mold within 30 days of conditioning for transplant), history of HIV infection, be pregnant or breast feeding, or be a woman of child-bearing potential without acceptable contraception. In addition, patients were excluded if they had a prior myeloablative allogeneic transplant or autologous transplant, had extensive prior therapy including >12 months alkylator therapy or >6 months alkylator therapy with extensive radiation, or received Y-90 ibritumomab (Zevalin) or I-131 tostumomab (Bexxar).

Treatment

Patients were treated with cyclophosphamide 60 mg/kg/day IV (days −8 and −7), fludarabine 25 mg/m^2/day IV (days −8, −7, and −6), and total body irradiation 165 cGy twice a day (days −4, −3, −2, and −1) with the infusion of two UCB units on day 0 (Figure 1A). GVHD immunoprophylaxis consisted of cyclosporine (targeting trough levels, 200–400 μg/l) on days −3 to +100 with a 10% taper per week thereafter and mycophenolate mofetil 15 mg/kg IV three times a day on days −3 to +45 if no GVHD was present on day +45.

Three UCB units were identified before study enrollment, with one as backup should the expanded product HSC835 not meet lot release. All units had a minimum dose of 1.5×10^7 nucleated cells/kg recipient body weight and were HLA matched at a minimum of four of six loci with the patient and between units, considering HLA A and B at antigen-level and HLA DRB1 at allele-level typing. Unit 1 was the unit with highest cell dose except in one instance, when the unit was HLA matched at a comparable cell dose. Units were disqualified if donor-specific cross-reactive anti-HLA antibodies were identified.

Unit Processing

All UCB units were thawed using standard methods (Rubinstein et al., 1995). On day −15, the lower-dosed unit was CD34 enriched using the CliniMACS

Cell Selection Device (Miltenyi) following manufacturer's instructions and placed in expansion media, and the CD34− fraction was cryopreserved. The expansion culture media consisted of SCF, FLT-3L, TPO, and IL-6 (all at 50 ng/ml) and SR-1 without the addition of antibiotics. Before lot release, the product was required to have a cell viability $\geq$ 70%, negative gram stain, endotoxin < 5 EU/kg, and negative in-process aerobic and anaerobic bacterial or fungal cultures (Table S1). On day 0, the unmanipulated unit was infused, followed 4 hr later by the infusion of HSC835. On day +1, the cryopreserved CD34 depleted cells were thawed and infused.

Dose Capping

The goal was to infuse the maximum dose achieved by the expansion culture. While the lowest acceptable dose of TNCs and CD34+ cells has been defined for UCB transplant, it is not known whether there is an upper limit above which expanded cell infusions might be associated with unacceptable toxicities. Therefore, a staggered dose-capping approach was adopted to limit the maximum number of cells in the HSC835 product and potentially identify the maximal tolerable dose (Figure 1A). Dose-limiting toxicity (DLT) was defined as $\geq$grade 3 common terminology criteria for adverse events (CTCAE) hypersensitivity reactions to HSC835 infusion or $\geq$grade 4 CTCAE organ toxicities observed within the first 30 days after transplant. Excessive toxicity was defined on the basis of the observed rate of Bearman grade III and IV adverse events in the Cord Blood Transplantation trial (Kurtzberg et al., 2008). To this end, a minimum of three adult and three pediatric (aged 10–17 years) patients had to be enrolled at a given dose level before moving to the next dose level if there were no DLTs. Because not all expansion cultures exceeded the maximum dose at a given level, a total of 15 patients (13 adults and 2 children) were treated at dose level 1 (3×10^7 to 9×10^7 cells/kg body weight) or below and two adults were treated at dose level 2 (9.1×10^7 to 27×10^7 cells/kg body weight). Three patients (one adult and two children) achieved levels of cell expansion that exceeded the dose permitted (i.e., not all available cells were infused).

Correlative Studies

Chimerism Analysis

Peripheral blood from the recipient was collected before transplant and approximately 7, 14, 21, 28, 60, 100, 180, and 365 days after transplant. Peripheral blood mononuclear cells were prepared by mixing 10 ml of peripheral blood with an equal volume of a dextran-and-saline solution (3% dextran in 0.9% NaCl) for 30 min at room temperature. The leukocyte-rich plasma (upper) layer was pelleted, and the red blood cells were lysed. CD3 and CD15/CD33 cell populations in the peripheral blood were isolated by fluorescence-activated cell sorting. Chimerism testing was performed by a DNA-based assay for short tandem repeat loci using standard techniques (Brunstein et al., 2007).

Cord Blood Product Analysis

Hematopoietic subset analyses based on surface antigen expression and CFU enumeration were performed as previously described (Nawrot et al., 2011).

Analysis of Telomeric DNA

DNA was analyzed for telomere length as described previously (Celli and de Lange, 2005). Mobilized peripheral blood from normal donors or UCB was CD34 enriched and then cultured with SCF, TPO, FLT-3L, and IL-6 with and without SR-1 (10 days for mobilized peripheral blood and 15 days for UCB).

T Cell Alloreactivity Assay

Residual cells in the wash supernatant were collected when the cord blood units were thawed and washed. CD19+ cells in the supernatant were transformed with Epstein-Barr virus, and the resulting lymphoblastoid cell lines (LCLs) were cultured as described (Rickinson et al., 1984). On days 14, 28, and 56 after transplantation, 10 ml of peripheral blood was obtained from patients. Peripheral blood mononuclear cells were isolated by density gradient separation and cryopreserved. IFN-γ-secreting CD8+ cells following a 5 hr stimulation period with LCL from each UCB unit, as well as third-party cells, were analyzed as described previously (Gutman et al., 2010).

Data Collection and Statistical Analysis

Clinical outcome data were prospectively collected, and endpoints were defined using standard criteria (Wagner et al., 2002; Przepiorka et al., 1995; Weisdorf et al., 2003). In addition, outcomes in patients treated with HSC835 were compared to 111 patients with acute leukemia and myelodysplastic syndrome aged 10–55 years treated with the same conditioning and post-transplant immune suppression (historical control arm). The two groups were similar in terms of recipient age, height, weight, gender, CMV serostatus, HLA match, ABO match, and Karnofsky status. While patients in the historical cohort were more likely to have had acute myeloid leukemia ($p = 0.04$) and been transplanted over a broader time period (2000–2014, $p < 0.001$), with 64% after 2006, these factors were not expected to affect the primary endpoint. However, as expected, recipients of the expanded product HSC835 received a graft containing greater numbers of nucleated and CD34+ cells and CFU-GM colonies relative to those in the historical comparison cohort ($p < 0.001$ each; Table 2). In terms of transplant outcomes, neutrophil and platelet recoveries were determined as the day after transplant to achieve an absolute neutrophil count (ANC) $\geq 0.5 \times 10^9$/l for 3 consecutive days and ANC $\geq 20.0 \times 10^9$/l for 7 consecutive days without transfusion, respectively. Categorical variables were compared using the Pearson chi-square statistic, while comparisons with smaller numbers were analyzed with Fisher's exact test. The general Wilcoxon test was used to analyze continuous parameters between groups. Continuous factors across UCB units are compared by the Wilcoxon sign-rank test for paired data (Snedecor and Cochran, 1989). The Kaplan-Meier method was used to analyze the probability of overall survival (Kaplan and Meier, 1958). The log-rank statistic was used to complete comparisons. Cumulative incidence treating non-event death as a competing risk was used to estimate the probabilities of neutrophil and platelet recovery and acute and chronic GVHD. The probability of regimen-related mortality was estimated by treating relapse as a competing risk (Lin, 1997). All p values were reported as two sided, and a p value < 0.05 was considered statistically significant. All analyses were conducted using SAS 9.3 (SAS Institute) and R 3.0.2.

SUPPLEMENTAL INFORMATION

Supplemental Information includes one figure and one table and can be found with this article online at http://dx.doi.org/10.1016/j.stem.2015.10.004.

AUTHOR CONTRIBUTIONS

J.E.W. and C.C.B. designed and coordinated the study. T.E.D. and C.L. performed the statistical analyses, with an independent review of the analyses by J.J. D.M. and D.S. oversaw the manufacture of HSC835. A.E.B., D.S., and M.P.C. designed and coordinated the correlative assays. J.E.W. wrote the manuscript, and all coauthors had access to the primary data and contributed to the final report.

ACKNOWLEDGMENTS

The project was supported by Novartis Corporation and a grant from the National Cancer Institute (P01 CA065493-20; J.E.W., C.G.B., T.E.D., D.M., B.R.B., J.T., and C.L.). We wish to thank Diane Kadidlo, Fran Rabe, and staff of the Cell Therapy Laboratory at the Molecular and Cellular Therapeutics Facility for their efforts in product manufacturing, development, and monitoring; Dr. Eros Lazzerini Denchi for his investigations on telomere length; Stefanie Hage of the University of Minnesota and Suzanne Maahs and Shazia Ali of the Novartis Institutes for Biomedical Research for their critical assistance in patient monitoring and moving the clinical trial forward; Albert E. Parker for performing the T cell alloreactivity assays; Stephanie Fiola for qualification of the media and cytokines; and the faculty, advanced practice providers, unit and clinic nurses, pharmacists, and social workers of the Blood and Marrow Transplant Program who cared for the 20 patients on this phase I clinical trial at the University of Minnesota Medical Center and Masonic Children's Hospital. Specific authors are employees and shareholders of Novartis (J.J. and C.C.B.) and the Genomics Institute of the Novartis Foundation (A.E.B. and M.P.C.).

Received: July 1, 2015
Revised: September 12, 2015
Accepted: October 19, 2015
Published: December 3, 2015

REFERENCES

Ballen, K.K., Gluckman, E., and Broxmeyer, H.E. (2013). Umbilical cord blood transplantation: the first 25 years and beyond. Blood *122*, 491–498.

Barker, J.N., Weisdorf, D.J., and Wagner, J.E. (2001). Creation of a double chimera after the transplantation of umbilical-cord blood from two partially matched unrelated donors. N. Engl. J. Med. *344*, 1870–1871.

Barker, J.N., Weisdorf, D.J., DeFor, T.E., Blazar, B.R., McGlave, P.B., Miller, J.S., Verfaillie, C.M., and Wagner, J.E. (2005). Transplantation of 2 partially HLA-matched umbilical cord blood units to enhance engraftment in adults with hematologic malignancy. Blood *105*, 1343–1347.

Boitano, A.E., Wang, J., Romeo, R., Bouchez, L.C., Parker, A.E., Sutton, S.E., Walker, J.R., Flaveny, C.A., Perdew, G.H., Denison, M.S., et al. (2010). Aryl hydrocarbon receptor antagonists promote the expansion of human hematopoietic stem cells. Science *329*, 1345–1348.

Brunstein, C.G., Barker, J.N., Weisdorf, D.J., DeFor, T.E., Miller, J.S., Blazar, B.R., McGlave, P.B., and Wagner, J.E. (2007). Umbilical cord blood transplantation after nonmyeloablative conditioning: impact on transplantation outcomes in 110 adults with hematologic disease. Blood *110*, 3064–3070.

Cairo, M.S., Wagner, E.L., Fraser, J., Cohen, G., van de Ven, C., Carter, S.L., Kernan, N.A., and Kurtzberg, J. (2005). Characterization of banked umbilical cord blood hematopoietic progenitor cells and lymphocyte subsets and correlation with ethnicity, birth weight, sex, and type of delivery: a Cord Blood Transplantation (COBLT) Study report. Transfusion *45*, 856–866.

Celli, G.B., and de Lange, T. (2005). DNA processing is not required for ATM-mediated telomere damage response after TRF2 deletion. Nat. Cell Biol. *7*, 712–718.

Cheuk, D.K. (2013). Optimal stem cell source for allogeneic stem cell transplantation for hematological malignancies. World J. Transplant. *3*, 99–112.

Cutler, C., Multani, P., Robbins, D., Kim, H.T., Le, T., Hoggatt, J., Pelus, L.M., Desponts, C., Chen, Y.B., Rezner, B., et al. (2013). Prostaglandin-modulated umbilical cord blood hematopoietic stem cell transplantation. Blood *122*, 3074–3081.

de Lima, M., McMannis, J., Gee, A., Komanduri, K., Couriel, D., Andersson, B.S., Hosing, C., Khouri, I., Jones, R., Champlin, R., et al. (2008). Transplantation of ex vivo expanded cord blood cells using the copper chelator tetraethylenepentamine: a phase I/II clinical trial. Bone Marrow Transplant. *41*, 771–778.

de Lima, M., McNiece, I., Robinson, S.N., Munsell, M., Eapen, M., Horowitz, M., Alousi, A., Saliba, R., McMannis, J.D., Kaur, I., et al. (2012). Cord-blood engraftment with ex vivo mesenchymal-cell coculture. N. Engl. J. Med. *367*, 2305–2315.

Delaney, C., Heimfeld, S., Brashem-Stein, C., Voorhies, H., Manger, R.L., and Bernstein, I.D. (2010). Notch-mediated expansion of human cord blood progenitor cells capable of rapid myeloid reconstitution. Nat. Med. *16*, 232–236.

Farag, S.S., Srivastava, S., Messina-Graham, S., Schwartz, J., Robertson, M.J., Abonour, R., Cornetta, K., Wood, L., Secrest, A., Strother, R.M., et al. (2013). In vivo DPP-4 inhibition to enhance engraftment of single-unit cord blood transplants in adults with hematological malignancies. Stem Cells Dev. *22*, 1007–1015.

Flores, A.I., McKenna, D.H., Montalbán, M.A., De la Cruz, J., Wagner, J.E., and Bornstein, R. (2009). Consistency of the initial cell acquisition procedure is critical to the standardization of CD34+ cell enumeration by flow cytometry: results of a pairwise analysis of umbilical cord blood units and cryopreserved aliquots. Transfusion *49*, 636–647.

Gutman, J.A., Turtle, C.J., Manley, T.J., Heimfeld, S., Bernstein, I.D., Riddell, S.R., and Delaney, C. (2010). Single-unit dominance after double-unit umbilical cord blood transplantation coincides with a specific CD8+ T-cell response against the nonengrafted unit. Blood *115*, 757–765.

Horwitz, M.E., Chao, N.J., Rizzieri, D.A., Long, G.D., Sullivan, K.M., Gasparetto, C., Chute, J.P., Morris, A., McDonald, C., Waters-Pick, B., et al. (2014). Umbilical cord blood expansion with nicotinamide provides long-term multilineage engraftment. J. Clin. Invest. *124*, 3121–3128.

Kaplan, E.L., and Meier, P. (1958). Nonparametric estimation from incomplete observations. J. Am. Stat. Assoc. *53*, 457–481.

Krenger, W., Blazar, B.R., and Holländer, G.A. (2011). Thymic T-cell development in allogeneic stem cell transplantation. Blood *117*, 6768–6776.

Kurtzberg, J., Prasad, V.K., Carter, S.L., Wagner, J.E., Baxter-Lowe, L.A., Wall, D., Kapoor, N., Guinan, E.C., Feig, S.A., Wagner, E.L., and Kernan, N.A.; COBLT Steering Committee (2008). Results of the Cord Blood Transplantation Study (COBLT): clinical outcomes of unrelated donor umbilical cord blood transplantation in pediatric patients with hematologic malignancies. Blood *112*, 4318–4327.

Lin, D.Y. (1997). Non-parametric inference for cumulative incidence functions in competing risks studies. Stat. Med. *16*, 901–910.

Lund, T.C., Boitano, A.E., Delaney, C.S., Shpall, E.J., and Wagner, J.E. (2015). Advances in umbilical cord blood manipulation—from niche to bedside. Nat. Rev. Clin. Oncol. *12*, 163–174.

Migliaccio, A.R., Adamson, J.W., Stevens, C.E., Dobrila, N.L., Carrier, C.M., and Rubinstein, P. (2000). Cell dose and speed of engraftment in placental/umbilical cord blood transplantation: graft progenitor cell content is a better predictor than nucleated cell quantity. Blood *96*, 2717–2722.

Naldini, L. (2011). Ex vivo gene transfer and correction for cell-based therapies. Nat. Rev. Genet. *12*, 301–315.

Nawrot, M., McKenna, D.H., Sumstad, D., McMannis, J.D., Szczepiorkowski, Z.M., Belfield, H., Grassman, E., Temples, T., Nielsen, D., Yuan, N., et al. (2011). 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 *51*, 2001–2005.

Page, K.M., Zhang, L., Mendizabal, A., Wease, S., Carter, S., Gentry, T., Balber, A.E., and Kurtzberg, J. (2011). 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. *17*, 1362–1374.

Politikos, I., and Boussiotis, V.A. (2014). The role of the thymus in T-cell immune reconstitution after umbilical cord blood transplantation. Blood *124*, 3201–3211.

Popat, U., Mehta, R.S., Rezvani, K., Fox, P., Kondo, K., Marin, D., McNiece, I., Oran, B., Hosing, C., Olson, A., et al. (2015). Enforced fucosylation of cord blood hematopoietic cells accelerates neutrophil and platelet engraftment after transplantation. Blood *125*, 2885–2892.

Przepiorka, D., Weisdorf, D., Martin, P., Klingemann, H.G., Beatty, P., Hows, J., and Thomas, E.D. (1995). 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transplant. *15*, 825–828.

Radtke, S., Görgens, A., Kordelas, L., Schmidt, M., Kimmig, K.R., Köninger, A., Horn, P.A., and Giebel, B. (2015). CD133 allows elaborated discrimination and quantification of haematopoietic progenitor subsets in human haematopoietic stem cell transplants. Br. J. Haematol. *169*, 868–878.

Ramirez, P., Wagner, J.E., DeFor, T.E., Blazar, B.R., Verneris, M.R., Miller, J.S., McKenna, D.H., Weisdorf, D.J., McGlave, P.B., and Brunstein, C.G. (2012). Factors predicting single-unit predominance after double umbilical cord blood transplantation. Bone Marrow Transplant. *47*, 799–803.

Reisner, Y., Gur, H., Reich-Zeliger, S., Martelli, M.F., and Bachar-Lustig, E. (2003). Hematopoietic stem cell transplantation across major genetic barriers: tolerance induction by megadose CD34 cells and other veto cells. Ann. N Y Acad. Sci. *996*, 72–79.

Rickinson, A.B., Rowe, M., Hart, I.J., Yao, Q.Y., Henderson, L.E., Rabin, H., and Epstein, M.A. (1984). T-cell-mediated regression of "spontaneous" and of Epstein-Barr virus-induced B-cell transformation in vitro: studies with cyclosporin A. Cell. Immunol. *87*, 646–658.

Rubinstein, P., Dobrila, L., Rosenfield, R.E., Adamson, J.W., Migliaccio, G., Migliaccio, A.R., Taylor, P.E., and Stevens, C.E. (1995). Processing and cryopreservation of placental/umbilical cord blood for unrelated bone marrow reconstitution. Proc. Natl. Acad. Sci. USA *92*, 10119–10122.

Rubinstein, P., Carrier, C., Scaradavou, A., Kurtzberg, J., Adamson, J., Migliaccio, A.R., Berkowitz, R.L., Cabbad, M., Dobrila, N.L., Taylor, P.E., et al. (1998). Outcomes among 562 recipients of placental-blood transplants from unrelated donors. N. Engl. J. Med. *339*, 1565–1577.

Scaradavou, A.; National Cord Blood Program, New York Blood Center (2010). Unrelated umbilical cord blood unit selection. Semin. Hematol. *47*, 13–21.

Smith, A.R., and Wagner, J.E. (2009). Alternative haematopoietic stem cell sources for transplantation: place of umbilical cord blood. Br. J. Haematol. *147*, 246–261.

Snedecor, G., and Cochran, W. (1989). Statistical Methods, ed 8th (Iowa State University Press).

Wagner, J.E., Barker, J.N., DeFor, T.E., Baker, K.S., Blazar, B.R., Eide, C., Goldman, A., Kersey, J., Krivit, W., MacMillan, M.L., et al. (2002). Transplantation of unrelated donor umbilical cord blood in 102 patients with malignant and nonmalignant diseases: influence of CD34 cell dose and HLA disparity on treatment-related mortality and survival. Blood *100*, 1611–1618.

Wagner, J.E., Jr., Eapen, M., Carter, S., Wang, Y., Schultz, K.R., Wall, D.A., Bunin, N., Delaney, C., Haut, P., Margolis, D., et al.; Blood and Marrow Transplant Clinical Trials Network (2014). One-unit versus two-unit cord-blood transplantation for hematologic cancers. N. Engl. J. Med. *371*, 1685–1694.

Weisdorf, D.J., Hurd, D., Carter, S., Howe, C., Jensen, L.A., Wagner, J., Stablein, D., Thompson, J., and Kernan, N.A. (2003). Prospective grading of graft-versus-host disease after unrelated donor marrow transplantation: a grading algorithm versus blinded expert panel review. Biol. Blood Marrow Transplant. *9*, 512–518.

Wong, W.M., Sigvardsson, M., Åstrand-Grundström, I., Hogge, D., Larsson, J., Qian, H., and Ekblom, M. (2013). Expression of integrin α2 receptor in human cord blood CD34+CD38−CD90+ stem cells engrafting long-term in NOD/SCID-IL2Rγ(c) null mice. Stem Cells *31*, 360–371.

Yahata, T., Ando, K., Miyatake, H., Uno, T., Sato, T., Ito, M., Kato, S., and Hotta, T. (2004). Competitive repopulation assay of two gene-marked cord blood units in NOD/SCID/gammac(null) mice. Mol. Ther. *10*, 882–891.