In Vivo DPP-4 Inhibition to Enhance Engraftment of Single-Unit Cord Blood Transplants in Adults with Hematological Malignancies

Sherif S. Farag,¹ Shivani Srivastava,¹ Steven Messina-Graham,² Jennifer Schwartz,¹
Michael J. Robertson,¹ Rafat Abonour,¹ Kenneth Cornetta,¹ Lisa Wood,¹
Angie Secrest,¹ R. Matthew Strother,^{1,3} David R. Jones,³ and Hal E. Broxmeyer²

Delayed engraftment is a significant limitation of umbilical cord blood (UCB) transplantation due to low stem cell numbers. Inhibition of dipeptidyl peptidase (DPP)-4 enhanced engraftment in murine transplants. We evaluated the feasibility of systemic DPP-4 inhibition using sitagliptin to enhance engraftment of single-unit UCB grafts in adults with hematological malignancies. Twenty-four patients (21–58 years) received myeloablative conditioning, followed by sitagliptin 600 mg orally days –1 to +2, and single UCB grafts day 0. Seventeen receiving red cell-depleted (RCD) grafts, matched at 4 ($n=10$) or 5 ($n=7$) of 6 human leucocyte antigen (HLA) loci with median nucleated cell dose $3.6 (2.5–5.2) \times 10^7/\text{kg}$, engrafted at median of 21 (range, 13–50) days with cumulative incidence of 94% (95% confidence interval, 84%–100%) at 50 days. Plasma DPP-4 activity was reduced to $23\% \pm 7\%$ within 2 h. Area under DPP-4 activity-time curve (AUC_A) correlated with engraftment; 9 of 11 with $\text{AUC}_A < 6,000$ activity·h engrafted within ≤ 21 days, while all 6 with higher AUC_A engrafted later ($P=0.002$). Seven patients receiving red cell replete grafts had 10-fold lower colony forming units after thawing compared with RCD grafts, with poor engraftment. Systemic DPP-4 inhibition was well tolerated and may enhance engraftment. Optimizing sitagliptin dosing to achieve more sustained DPP-4 inhibition may further improve outcome.

Introduction

UMBILICAL CORD BLOOD (UCB) is an established hematopoietic stem cell (HSC) source for transplantation [1]. Delayed engraftment, however, remains a significant limitation due to low cell doses in UCB units. Strategies to overcome this limitation include use of multiple cord blood units [2,3], ex vivo expansion of UCB stem cells [4], and co-infusion of third party haploidentical CD34+ cells [5,6], but technical obstacles, complexity of procedures, and/or economic considerations may limit their widespread use.

We focused on a simple, inexpensive approach to enhance UCB engraftment. Stromal-derived factor (SDF)-1 α , and its receptor CXCR4 play key roles in homing/nurturing of hematopoietic stem/progenitor cells [7,8]. Dipeptidyl peptidase (DPP)-4 regulates the chemotaxis and engraftment effects of SDF-1 α [9,10], expresses as surface CD26 on hematopoietic stem/progenitors and other cell types [9,10], and is also present in a catalytically active soluble form in plasma [11]. DPP-4 cleaves N-terminal dipeptides from a variety of substrates including SDF-1 α at the penultimate proline or

alanine [12,13]. Truncated SDF-1 (aa 3–68) cannot activate CXCR4, but antagonizes signals from full-length SDF-1 α [9,10]. We demonstrated that inhibition of DPP-4 enhances chemotaxis of human UCB CD34+ cells to a SDF-1 α gradient [9]. Further, inhibition or deletion of DPP-4 enhances engraftment of mouse bone marrow cells [10] and human UCB CD34+ cells [13–15] in mice. Finally, intravenous or oral administration of a DPP-4 inhibitor to lethally irradiated mice also enhances engraftment of untreated mouse marrow cells [16–18]. Based on these data, we conducted a pilot trial evaluating feasibility and safety, and potential efficacy of systemic inhibition of DPP-4 using orally administered sitagliptin, an FDA approved DPP-4 inhibitor, to enhance engraftment of single-unit UCB transplants in adults with high-risk hematological malignancies.

Design and Methods

Patients

Eligible patients had high-risk acute myeloid leukemia (AML) or acute lymphoblastic leukemia in complete remission

¹Division of Hematology-Oncology, Department of Medicine, Indiana University School of Medicine, Indianapolis, Indiana.

²Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, Indiana.

³Department of Medicine, Clinical Pharmacology, Indiana University School of Medicine, Indianapolis, Indiana.

(CR) or relapse, refractory aggressive non-Hodgkin's lymphoma (NHL), or refractory chronic myelogenous leukemia. Patients were aged 18–59 years, had a Karnofsky performance status $\geq 70\%$, cardiac left ventricular ejection fraction $>45\%$, gas transfer diffusing capacity of lung for carbon monoxide $>50\%$, creatinine clearance >60 mL/min/1.73 m², and serum bilirubin $<2 \times$ upper limit of normal. Treatment was restricted to patients lacking human leucocyte antigen (HLA)-identical related or well-matched volunteer unrelated donors, or if the patient's disease was unstable, matched adult donor cells could not be procured within 4 weeks. The trial was approved by the Indiana University Institutional Review Board and was registered at ClinicalTrials.gov (NCT00862719). All patients gave written informed consent.

Selection of UCB grafts

Single UCB units were used as we felt that this was the only way to definitively investigate potential efficacy of effects on HSC engraftment. UCB units were matched at 4 or more of 6 HLA loci (HLA-A, -B, -DRB1) with recipient, and they contained a minimum nucleated cell (NC) dose of 2.5×10^7 /kg recipient body weight before freezing. Donor units with the best HLA-match were preferred, and within an HLA match level units with greatest NC numbers were selected.

Treatment

Patients received 13.2 Gy total body irradiation in 8 fractions on days –7 to –4, cyclophosphamide 60 mg/kg on days –3 and –2, and rabbit antithymocyte globulin (ATG) 2.5 mg/kg on days –4 to –2. To reduce the risk of infection, the protocol replaced ATG with fludarabine 30 mg/m² on days –4 to –2 after the first 11 patients were enrolled. UCB units were thawed and washed as previously described [19] and infused on day 0. Sirolimus and tacrolimus were administered for graft-versus-host disease (GvHD) prophylaxis, as previously reported [20]. Sitagliptin (Januvia®; Merck & Co., Inc., Whitehouse Station, NJ) 600 mg every 24 h was orally administered from days –1 to +2. The dose of sitagliptin was based on pharmacodynamic studies in healthy volunteers demonstrating $>90\%$ plasma DPP-4 inhibition that was sustained for more than 24 h following 600 mg doses [21]. Filgrastim (5 µg/kg/day subcutaneously) was started on day +5 and continued until neutrophil recovery. Patients received antibiotic prophylaxis against bacterial, fungal, and viral infections.

Pharmacokinetic and pharmacodynamic studies

Blood was collected for sitagliptin levels and plasma DPP-4 activity at baseline, and then at multiple time points between 0.5 and 24 h after the first dose, and between 2 and 24 h after subsequent doses for plasma DPP-4 activity. Sitagliptin concentrations were quantified by liquid–liquid extraction and high-performance liquid chromatography tandem mass spectrometry (API 3200; Applied Biosystems, Toronto, Canada) using fluoxetine as the internal standard. The lower limit of quantification was 0.5 ng/mL. Plasma DPP-4 activity was assayed using the DPPIV-Glo Protease Assay (Promega, Madison, WI), according to manufacturer's instructions.

Definitions and statistical analysis

Engraftment was defined as achieving an absolute neutrophil count (ANC) $>0.5 \times 10^9$ /L for 3 consecutive days with donor derived cells, confirmed by short tandem repeat loci analysis of blood or bone marrow [22]. Platelet recovery was defined as achieving platelets $>20 \times 10^9$ /L without transfusion for 7 consecutive days. Acute and chronic GvHD were graded using standard criteria [23,24], and toxicity using the NCI Common Terminology Criteria for Adverse Events v3.0.

Cumulative incidences of neutrophil and platelet engraftment were calculated from day 0 until neutrophil engraftment and platelet recovery, respectively, with death prior to recovery a competing risk. Cumulative incidence of non-relapse mortality (NRM) was calculated from day 0 to death from any cause other than relapse, with death due to relapse a competing risk. Event-free survival (EFS) and overall survival (OS) were secondary endpoints, calculated using the Kaplan–Meier method [25]. The independent association of the extent of plasma DPP-4 inhibition, as measured by area under the curve of residual plasma DPP-4 activity, with myeloid recovery earlier than the median time to engraftment was studied using multivariable logistic regression analysis using a backward selection strategy, controlling for baseline clinical characteristics known to affect engraftment [26,27], including age, NC dose, disease status at time of transplantation, and degree of HLA mismatch. For all analyses, a 2-sided α -value of 0.05 was considered significant.

Results

Patients and UCB grafts

Twenty-four consecutive patients undergoing UCB transplantation at Indiana University Medical Center between December 2009 and December 2011 were enrolled in this study (Table 1). Seventeen patients received red cell-depleted (RCD) and 7 received red cell-replete, plasma-depleted (PD) UCB grafts. As shown in Table 2, there was no significant difference between RCD and PD units in pre-freezing or post-thawing NC doses, cell viability and recovery, and CD34+ cell dose infused. However, median colony forming unit (CFU) numbers after thawing were almost 10-fold lower in PD compared with RCD grafts ($P=0.0002$).

Engraftment and hematopoietic recovery

CFU numbers have been reported to be strongly associated with engraftment [28]. As CFU numbers in PD units were much lower than in RCD units, and could therefore confound evaluation of effects of DPP-4 inhibition, engraftment kinetics were separately analyzed for the RCD and PD groups. Of 17 patients transplanted with RCD units, 1 died on day 29 due to sepsis with beginning neutrophil recovery (ANC 0.3×10^9 /L) at time of death. All remaining 16 patients survived beyond day +30 and engrafted with donor-derived hematopoiesis by day +50. Median time to engraftment was 21 (range, 13–50) days (Fig. 1a), and the cumulative incidence of neutrophil engraftment by day +30 was 88% [95% confidence interval (CI) 74%–100%], and 94% (95% CI, 84%–100%) by day +50. The median percent donor chimerism at

TABLE 1. PATIENT CHARACTERISTICS

	All patients (n=24)	RCD (n=17)	PD (n=7)
Age, median (range) years	39 (21–58)	40 (21–58)	35 (21–46)
Weight, median (range) kg	74 (47–119)	68 (47–103)	85 (67–119)
M/F, n	8/16	5/12	3/4
Diagnosis, n			
AML			
CR1/CR2	7	7	0
Rel Ref	8	7	1
ALL			
CR1/CR2	5	0	5
NHL			
Rel Ref	3	2	1
CML (late chronic phase) ^a	1	1	0
HLA-match, n			
4 of 6	12	10	2
5 of 6	12	7	5

^aCML patient in late chronic phase failed treatment with 3 tyrosine kinase inhibitors.

AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; NHL, non-Hodgkin's lymphoma; CML, chronic myelogenous leukemia; CR, complete remission; Rel Ref, relapsed and/or refractory; UCB, umbilical cord blood; RCD, red cell depleted UCB graft; PD, plasma depleted UCB grafts; HLA, human leucocyte antigen.

time of engraftment was 100% (range, 74%–100%). Median time to platelet recovery to $>20 \times 10^9/L$ was 77 (range, 48–220) days, with a cumulative incidence of 71% (95% CI 52%–96%) by day +100 (Fig. 1b). Three of the 17 patients receiving RCD UCB units died on days +29, +36, and +56 due to sepsis, cytomegalovirus (CMV) pneumonitis, and relapse of AML, respectively, before platelet recovery. All 14 remaining patients recovered platelet counts, although precise evaluation of platelet recovery was difficult in 4 patients because of thrombotic microangiopathy ($n=3$) or continuing need for platelet transfusions for gastrointestinal bleeding ($n=1$). Of the patients who received RCD grafts, the median time of neutrophil engraftment for patients who received ATG ($n=7$) was 19 (range, 13–29) days, compared with a median of 23 (range, 16–50) days for patients who received fludarabine as part of the preparative regimen ($n=10$) ($P=0.20$; Log-rank test). Thus, while the trend was for longer time to myeloid engraftment with fludarabine, there was no statistical difference between patients who received ATG or flu-

darabine. Engraftment was durable in all patients and no cases of secondary graft failure were observed.

For the 7 patients receiving PD grafts, 1 died of sepsis on day +105 without engraftment. Median time to neutrophil engraftment was 36 (range, 26–99) days, with 2 patients engrafting by day +30. The longer time to myeloid engraftment with PD grafts likely reflects the much lower CFU numbers infused in these units. Only 2 of 7 patients receiving PD units achieved platelets $>20 \times 10^9/L$ on days +80 and +102.

Toxicity, NRM, and GvHD

Grade 3–4 toxicity for all 24 patients included gastrointestinal (vomiting, diarrhea, and oral mucositis) ($n=12$), acute renal insufficiency ($n=2$), sinusoidal obstruction syndrome ($n=3$), thrombotic microangiopathy ($n=3$), acute cholecystitis ($n=1$), gastrointestinal bleeding ($n=1$), and posterior reversible encephalopathy syndrome that resolved on discontinuation of tacrolimus and sirolimus ($n=1$). No patient developed hypoglycemia.

The cumulative incidences of NRM at 6 months and 1 year for patients transplanted with RCD grafts were both 20% (95% CI 7%–57%) (Fig. 2a). Of 17 patients transplanted with RCD units, 4 died of infection, including *Staphylococcus aureus* pneumonia (day +29), CMV pneumonitis (day +36), *Klebsiella pneumoniae* septicemia (day +137), and pulmonary aspergillosis in context of corticosteroid therapy for chronic GvHD (day +606). Of 7 patients transplanted with PD grafts, 6 died of infectious complications, including HHV-6 ($n=1$), adenovirus ($n=1$), toxoplasmosis ($n=1$), and bacterial sepsis ($n=3$).

Among 17 patients receiving RCD grafts, only 1 developed grade II acute GvHD on day +259 while tapering immune suppression. Of 11 patients who survived beyond day +100, 1 developed de novo extensive stage chronic GvHD and died of infection on day +606. No GvHD was observed among patients transplanted with PD units.

EFS and OS

Response, EFS and OS were all secondary endpoints of the study. Overall, all 11 patients with active leukemia ($n=8$) or NHL ($n=3$) at time of transplantation achieved CR. Among the 17 patients receiving RCD units, 4 with refractory AML at transplantation died of relapse between days +55 to +159. After a median follow-up of 259 (range, 84–736) days for surviving patients, median EFS and OS for patients receiving RCD grafts were both 606 days (Fig. 2b, c). One-year EFS was 53% (95% CI, 33%–87%) and 1-year

TABLE 2. UMBILICAL CORD BLOOD GRAFT CHARACTERISTICS

	All (n=24)	RCD (n=17)	PD (n=7)	P-value ^a
Pre-freeze NC ($\times 10^7/kg$)	3.5 (2.5–5.2)	3.6 (2.5–5.2)	2.9 (2.7–4.5)	0.34
Post-thaw NC ($\times 10^7/kg$)	2.3 (1.4–3.8)	2.4 (1.4–3.8)	2.2 (1.4–3.2)	0.36
Post-thaw viability (%)	85 (65–92)	82 (66–90)	85 (65–92)	0.59
Paired post-thaw NC recovery (%)	82 (62–106)	81 (65–106)	83 (62–96)	0.33
Post-thaw CD34+ cells ($\times 10^5/kg$)	1.0 (0.1–8.3)	1.0 (0.1–8.3)	0.7 (0.5–3.0)	0.13
CFU ($\times 10^4/kg$)	1.1 (0.05–4.9)	1.7 (0.4–4.9)	0.2 (0.05–1.1)	0.002

All results presented as median (range).

^aP-value reflects 2-sided significance level of comparison between RCD and PD grafts (Mann–Whitney test).

NC, nucleated cell; CFU, colony forming unit.

FIG. 1. Engraftment kinetics. (a) Cumulative incidence of neutrophil engraftment. (b) Cumulative incidence of platelet engraftment. Cumulative incidences are shown for red cell-depleted (RCD) grafts (solid line) and plasma-depleted (PD) grafts (dashed line).

OS was 59% (95% CI, 38%–92%), with 9 of 17 patients alive and disease-free beyond days +84 to +736. Of 7 patients receiving PD grafts, only 1, engrafting on day +26, remains alive with normal blood counts and ongoing CR at day +308.

Sitagliptin pharmacokinetics and pharmacodynamics

Although there was no significant difference in extent of plasma DPP-4 inhibition between patients receiving RCD

FIG. 2. Mortality and survival for patients receiving RCD umbilical cord blood (UCB) transplants. (a) Cumulative incidence of non-relapse mortality (solid line) and mortality due to relapse (dashed line) shown as competing events. (b) Event-free survival. (c) Overall survival.

and PD grafts ($P > 0.1$, all time points), only the results for RCD transplants are presented because we wished to correlate DPP-4 inhibition with engraftment without the confounding effect of low CFUs associated with PD grafts. As shown in Fig. 3a, maximal inhibition of plasma DPP-4 activity occurred 2–4 h after dosing. Mean ($\pm$ standard error) DPP-4 activity was $23\% \pm 7\%$ of baseline 2 h after sitagliptin administration. However, DPP-4 inhibition was not sustained, returning to 80% or more of baseline by 16 h after each dose.

To correlate plasma DPP-4 inhibition with engraftment, the sum of areas under the percent residual activity of DPP-4 versus time curve (AUC_A) for each day of sitagliptin dosing was calculated. There was no significant difference between the total AUC_A for patients receiving RCD grafts (median 5,169 h·activity, range 2,932–7,530 h·activity) and that for patients receiving PD grafts (median 5,841 h·activity, range 3,198–9,042 h·activity; $P = 0.37$). For patients receiving RCD UCB grafts, the total AUC_A of patients engrafting within ≤ 21 (range, 13–20) days was compared with that of patients taking longer to engraft (range, 22–50 days) (Fig. 3b). Median

total AUC_A for patients engrafting in ≤ 21 days was 4,189 (range, 3,563–5,903) h·activity, compared with 6,729 (range, 2,932–6,155) h·activity for patients engrafting > 21 days ($P = 0.024$; Mann–Whitney test). Nine of eleven patients with total $AUC_A < 6,000$ h·activity engrafted before day +21, while all 6 with higher total AUC_A engrafted later ($P = 0.002$; Fisher's exact test). On multivariable logistic regression analysis, a lower AUC_A was the only significant factor associated with engraftment earlier than 17 days, after adjusting for age, TNC dose, HLA mismatch (1 vs. 2 mismatches), and presence of active leukemia at time of transplantation [odds ratio 0.25 (95%CI 0.10–0.89); $P = 0.03$].

Plasma sitagliptin concentration profile for the 17 patients receiving RCD grafts is summarized in Fig. 3c. Median maximum concentration (C_{max}) of 1,690 (range, 119–4,813) ng/mL was achieved at median T_{max} of 2 (range, 0.5–4.8) h, and the median elimination half-life ($t_{1/2}$) was 3.9 (range, 3.0–8.5) h. Median plasma sitagliptin $AUC_{0-\infty}$ was 11,661 (range, 711–31,766) ng/mL·h. Overall, greater plasma DPP-4 inhibition was observed when higher plasma sitagliptin concentrations were achieved (Fig. 3d). There was no

FIG. 3. Sitagliptin pharmacokinetics and pharmacodynamics. (a) Plasma dipeptidyl peptidase (DPP)-4 activity following sitagliptin dosing. DPP-4 activity is shown as a percentage of baseline activity. Data represent mean values $\pm$ standard error for 17 patients who received RCD grafts. (b) Relationship between area under the plasma DPP-4 activity versus time curve (AUC_A) and engraftment. Patients engrafting within 21 days had significantly lower AUC_A compared with those engrafting beyond 21 days ($P = 0.024$; 2-sided Mann–Whitney test). (c) Plasma sitagliptin concentration–time profile. Data represent mean $\pm$ standard error of plasma sitagliptin concentration after the first dose on day –1 for the 17 patients who received RCD grafts. (d) Relationship between sitagliptin concentrations achieved and percent inhibition of plasma DPP-4 during the first 24 h after dosing on day –1.

significant difference in $AUC_{0-\infty}$ between patients who engrafted within 21 days and those engrafting later ($P=0.41$; data not shown).

Discussion

We describe feasibility and safety in the first clinical trial investigating DPP-4 inhibition to enhance engraftment following single-unit UCB transplantation in adults with hematological malignancies. The observed median time to engraftment of 21 days using single UCB units compares favorably with a majority of reports where comparable cell doses were infused. Further, our result is especially noteworthy since most of our recipients received 4/6 HLA matched grafts and most had active marrow involvement by leukemia at the time of transplantation, which is a significant factor associated with a longer time to engraftment [27].

Multiple studies have demonstrated adverse effects of low cell dose on engraftment, transplant-related mortality, and survival [29–32]. A New York Blood Center study of over 600 myeloablative single-unit UCB transplants showed that to achieve 1-year OS of >50%, NC dose must be $\geq 2.5 \times 10^7$ /kg for 5/6 HLA-matched, and $\geq 5 \times 10^7$ /kg for 4/6 matched units [33]. However, only a minority of adults will find UCB units based on these recommendations. Therefore, enhancing engraftment of lower cell doses will likely significantly impact outcomes.

Although the optimal approach to enhance engraftment by inhibiting DPP-4 (ex-vivo or systemic) is uncertain [16–18], we used oral sitagliptin to avoid the complexity and regulatory hurdles associated with ex vivo manipulation of

UCB, and developed a simple, inexpensive method easily usable by transplantation centers. Sitagliptin is a selective inhibitor of DPP-4 approved by the FDA for treatment of type II diabetes mellitus [34]. In this study, sitagliptin used at 600 mg/day was well tolerated and was not associated with any apparent additional toxicity than might be expected in the setting of myeloablative conditioning and UCB transplantation. Importantly, inhibition of DPP-4 yielded favorable engraftment kinetics, with a median time to neutrophil engraftment of 21 days and a cumulative incidence of engraftment of 94% (95% CI 84%–100%) at 50 days in patients receiving RCD grafts. Our results compare favorably with major published series where predominantly adult patients with hematological malignancies received either single or double UCB grafts [27,35–39], as reviewed in Table 3. As shown, most reported median times to engraftment have been 22 days or longer with the exception of the series reported by Sanz et al. [27], which included a significant number of pediatric patients and only 21% of patients had active disease at the time of transplantation. In the latter report [27], active disease at the time of transplantation was the single most important factor adversely affecting engraftment. Nonetheless, there is significant limitation in evaluating the engraftment kinetics observed in our study relative to those in published reports because of significant differences in important variables that could affect engraftment, including cell dose, degree of HLA match, disease phase, and age of patients treated. However, in the largest, recently updated reported series analyzing engraftment following 1,061 single-unit UCB transplants in patients with hematological malignancies, including over 300 patients with

TABLE 3. CHARACTERISTICS AND REPORTED ENGRAFTMENT TIMES IN MAJOR PUBLISHED SERIES OF PREDOMINANTLY ADULT PATIENTS WITH HEMATOLOGICAL MALIGNANCIES UNDERGOING UMBILICAL CORD BLOOD TRANSPLANTATION

	Disease	N	No. HLA mismatches	Median (range) age (years)	Cell dose ($\times 10^7$ /kg)	Median time to engraftment (days)	CI of engraftment
Rocha et al. [35]	AML, ALL	98	0–3	24.5 (15–55)	2.3 (0.9–6)	26	75% at 60 days
Laughlin et al. [36]	AML, ALL, MDS, CML (43% with active disease)	150	0–2	N/A (16–60)	2.2 (1–6.5)	27	70% at 100 days
Takahashi et al. [37]	AML, ALL, CML, MDS, NHL	100	0–3	38 (16–55)	2.43 (1.1–5.3) 93% received > 2.0×10^7 /kg	22	91% at 60 days
Eapen et al. [38]	AML, ALL (25% in active relapse)	165	0–2	28 (All >16 years)	3.6 (2.5–9.3)	24	80% at 42 days
Brunstein et al. [39] ^a	AML, ALL, MDS, CML (all patients with acute leukemia in CR)	128	0–2	25 (10–46)	4.0 (2–30)	26	84% at 45 days
Sanz et al. [27]	AML, ALL, NHL, HD, MDS (21% with active disease)	88	0–2	28 (1–52)	2.4 (1.4–32)	19	94% at day 45

^aDouble UCB units were employed in the series with cell doses reflect the combined cell dose of the 2 UCB units used. MDS, myelodysplasia; HD, Hodgkin's disease; N/A, not available from publication; CI, confidence interval.

highly comparable features to patients included in our trial in terms of cell dose range ($2.5\text{--}4.9 \times 10^7/\text{kg}$), degree of HLA-mismatch (1–2 mismatches), and type of graft used (RCD), the median time of neutrophil engraftment was ~ 28 days, with a cumulative incidence of engraftment of less than 80% at 100 days [26]. Notably, in this updated series, the median time to engraftment was not substantially improved despite inclusion of almost 500 additional patients who were treated more recently [26,30].

While DPP-4 inhibition might have had a positive impact on neutrophil engraftment, the effects on platelet recovery are less certain and will need further study. In at least 4 patients, time to recovery of platelets to $>20 \times 10^9/\text{L}$ was difficult to assess because of increased platelet consumption due to thrombotic microangiopathy (likely related to the use of sirolimus and tacrolimus for GvHD prophylaxis), and need for repeated transfusions for gastrointestinal bleeding. Better platelet engraftment kinetics may have become apparent in the absence of these complications.

A notable observation in our study is the very low incidence of GvHD. Only 1 of 17 patients receiving RCD grafts developed acute GvHD, and 1 of 11 evaluable patients developed chronic GvHD; frequencies much lower than previously reported even when ATG has been used [3,36,38]. Further studies with more patients are required to confirm this observation.

Seven patients receiving PD grafts in our study had worse engraftment and long-term outcome, likely related to very low CFU numbers following thawing and washing of these units; levels likely too low to properly test effects of DPP-4 inhibition on engraftment. The very low CFU numbers obtained from PD grafts may be related to our washing method used being suboptimal for these types of units [40].

While in vivo DPP-4 inhibition was feasible, improvement of this approach may be possible. We did not achieve the expected sustained inhibition of plasma DPP-4 activity as published in healthy volunteers ($>90\%$ plasma DPP-4 inhibition for 24 h) [21]. Importantly, plasma DPP-4 inhibition was significantly associated with engraftment indicating that plasma DPP-4 activity may be a good surrogate for bone marrow DPP-4 activity. The reasons for suboptimal DPP-4 inhibition remain unclear because sitagliptin pharmacokinetics were similar to those previously reported [21]. DPP-4 may be upregulated, or more enzyme released from a cellular pool [18] following cell death associated with myeloablative chemoradiotherapy in ways not previously appreciated. Sitagliptin given 2 or 3 times per day may result in more sustained DPP-4 inhibition, which may further improve engraftment kinetics. In addition, we recently showed that DPP-4 also regulates potency of granulocyte-macrophage colony stimulating factor, granulocyte-colony stimulating factor, interleukin-3, and erythropoietin, all of which have the truncation site for the enzyme, and inhibition or deletion of DPP-4 enhances cytokine activities and in vivo hematopoietic recovery after cytotoxic stress [18], suggesting longer administration of sitagliptin may further enhance engraftment.

Other strategies to overcome limitations of low cell dose and enhance engraftment have been suggested [41]. Transplantation of double UCB units has led to increased numbers of adults receiving transplants without evidence that engraftment is significantly enhanced compared with single UCB units [3,42]. Double UCB units significantly increase

cost and demand on cord banks to collect more units. Direct intraosseous infusion has been investigated [43], although it remains unclear whether this improves engraftment, and the need for general anesthesia may be difficult for some patients. Although co-infusion of purified, mobilized haploidentical CD34 cells [5,6], and co-infusion of ex vivo Notch ligand-expanded UCB stem cells together with unmanipulated cord cells [4], may somewhat improve engraftment kinetics, these approaches are costly and may be prohibitive for some centers. Approaches that enhance homing, including ex vivo fucosylation [44], or treatment of UCB cells with prostaglandin E_2 [45,46], may offer potential synergy with DPP-4 inhibition.

Our results indicate that systemic DPP-4 inhibition may be a reasonable strategy for enhancing engraftment of single-unit UCB transplants, and may offer advantages over other approaches in simplicity and affordability. Improved dosing of sitagliptin to achieve more sustained DPP-4 inhibition, and also for a longer duration to enhance effects on hematopoietic cytokines, may result in enhanced efficacy, and requires further clinical investigation in larger trials.

Acknowledgments

Parts of these studies were supported by grants from the V Foundation for Cancer Research (S.S.F.), and Public Service multi-PI R01HL112669 from the National Institutes of Health (NIH) of the United States (H.E.B. and S.S.F.). S.M.-G. was supported as a predoctoral student on an underrepresented minority grant GM079657 (H.E.B.) from the NIH. Pharmacological work was performed by the Clinical Pharmacology Analytical Core Laboratory of the Indiana University Melvin and Bren Simon Cancer Center and supported by Institutional grant P30 CA082709.

Author Disclosure Statement

H.E.B. is a founder of Cord: Use Family Cord Blood Bank and serves on the medical scientific advisory board of the Cord: Use Public Cord Blood Bank.

References

1. Broxmeyer HE and FO Smith. (2009). Cord blood hematopoietic cell transplantation. In: *Thomas' Hematopoietic Cell Transplantation, 4th Edition*. Appelbaum FR, SJ Forman, RS Negrin and KG Blume, eds. Wiley Blackwell, Malden, MA, pp 559–575.
2. Barker JN, DJ Weisdorf, TE DeFor, BR Blazar, PB McGlave, JS Miller, CM Verfaillie and JE Wagner. (2005). Transplantation of 2 partially HLA-matched umbilical cord blood units to enhance engraftment in adults with hematologic malignancy. *Blood* 105:1343–1347.
3. Rocha V, A Crotta, A Ruggeri, D Purtill, K Boudjedir, AL Herr, I Ionescu and E Gluckman. (2010). Double cord blood transplantation: extending the use of unrelated umbilical cord blood cells for patients with hematological diseases. *Best Pract Res Clin Haematol* 23:223–229.
4. Delaney C, S Heimfeld, C Brashem-Stein, H Voorhies, RL Manger and ID Bernstein. (2010). Notch-mediated expansion of human cord blood progenitor cells capable of rapid myeloid reconstitution. *Nat Med* 16:232–236.

5. Bautista G, JR Cabrera, C Regidor, R Fores, JA Garcia-Marco, E Ojeda, I Sanjuan, E Ruiz, I Krsnik, et al. (2009). Cord blood transplants supported by co-infusion of mobilized hematopoietic stem cells from a third-party donor. *Bone Marrow Transplant* 43:365–373.
6. Liu H, ES Rich, L Godley, O Odenike, L Joseph, S Marino, J Kline, V Nguyen, J Cunningham, et al. (2011). Reduced-intensity conditioning with combined haploidentical and cord blood transplantation results in rapid engraftment, low GVHD, and durable remissions. *Blood* 118:6438–6445.
7. Peled A, I Petit, O Kollet, M Magid, T Ponomaryov, T Byk, A Nagler, H Ben-Hur, A Many, et al. (1999). Dependence of human stem cell engraftment and repopulation of NOD/SCID mice on CXCR4. *Science* 283:845–848.
8. Broxmeyer HE, S Cooper, L Kohli, G Hangoc, Y Lee, C Mantel, DW Clapp and CH Kim. (2003). Transgenic expression of stromal cell-derived factor-1/CXC chemokine ligand 12 enhances myeloid progenitor cell survival/antiapoptosis *in vitro* in response to growth factor withdrawal and enhances myelopoiesis *in vivo*. *J Immunol* 170:421–429.
9. Christopherson KW, 2nd, G Hangoc and HE Broxmeyer. (2002). Cell surface peptidase CD26/dipeptidylpeptidase IV regulates CXCL12/stromal cell-derived factor-1 alpha-mediated chemotaxis of human cord blood CD34+ progenitor cells. *J Immunol* 169:7000–7008.
10. Christopherson KW, 2nd, G Hangoc, CR Mantel and HE Broxmeyer. (2004). Modulation of hematopoietic stem cell homing and engraftment by CD26. *Science* 305:1000–1003.
11. Durinx C, AM Lambeir, E Bosmans, JB Falmagne, R Berghmans, A Haemers, S Scharpe and I De Meester. (2000). Molecular characterization of dipeptidyl peptidase activity in serum: soluble CD26/dipeptidyl peptidase IV is responsible for the release of X-Pro dipeptides. *Eur J Biochem* 267:5608–5613.
12. Mentlein R. (1999). Dipeptidyl-peptidase IV (CD26)—role in the inactivation of regulatory peptides. *Regul Pept* 85:9–24.
13. Campbell TB, G Hangoc, Y Liu, K Pollok and HE Broxmeyer. (2007). Inhibition of CD26 in human cord blood CD34+ cells enhances their engraftment of nonobese diabetic/severe combined immunodeficiency mice. *Stem Cells Dev* 16:347–354.
14. Christopherson KW, 2nd, LA Paganessi, S Napier and NK Porecha. (2007). CD26 inhibition on CD34+ or lineage-human umbilical cord blood donor hematopoietic stem cells/hematopoietic progenitor cells improves long-term engraftment into NOD/SCID/Beta2null immunodeficient mice. *Stem Cells Dev* 16:355–360.
15. Kawai T, U Choi, PC Liu, NL Whiting-Theobald, GF Linton and HL Malech. (2007). Diprotin A infusion into nonobese diabetic/severe combined immunodeficiency mice markedly enhances engraftment of human mobilized CD34+ peripheral blood cells. *Stem Cells Dev* 16:361–370.
16. Broxmeyer HE, G Hangoc, S Cooper, T Campbell, S Ito and C Mantel. (2007). AMD3100 and CD26 modulate mobilization, engraftment, and survival of hematopoietic stem and progenitor cells mediated by the SDF-1/CXCL12-CXCR4 axis. *Ann N Y Acad Sci* 1106:1–19.
17. Campbell TB and HE Broxmeyer. (2008). CD26 inhibition and hematopoiesis: a novel approach to enhance transplantation. *Front Biosci* 13:1795–1805.
18. Broxmeyer HE, J Hoggatt, HA O'Leary, C Mantel, BR Chitteti, S Cooper, S Messina-Graham, G Hangoc, SS Farag, et al. (2012). Dipeptidylpeptidase 4 negatively regulates colony stimulating factor activity and stress hematopoiesis. *Nat Med* 18:1786–1796.
19. Rubinstein P, L Dobrila, RE Rosenfield, JW Adamson, G Migliaccio, AR Migliaccio, PE Taylor and CE Stevens. (1995). Processing and cryopreservation of placental/umbilical cord blood for unrelated bone marrow reconstitution. *Proc Natl Acad Sci U S A* 92:10119–10122.
20. Cutler C, HT Kim, E Hochberg, V Ho, E Alyea, SJ Lee, DC Fisher, D Miklos, J Levin, et al. (2004). Sirolimus and tacrolimus without methotrexate as graft-versus-host disease prophylaxis after matched related donor peripheral blood stem cell transplantation. *Biol Blood Marrow Transplant* 10:328–336.
21. Bergman AJ, C Stevens, Y Zhou, B Yi, M Laethem, M De Smet, K Snyder, D Hilliard, W Tanaka, et al. (2006). Pharmacokinetic and pharmacodynamic properties of multiple oral doses of sitagliptin, a dipeptidyl peptidase-IV inhibitor: a double-blind, randomized, placebo-controlled study in healthy male volunteers. *Clin Ther* 28:55–72.
22. Nakamura Y, M Leppert, P O'Connell, R Wolff, T Holm, M Culver, C Martin, E Fujimoto, M Hoff, et al. (1987). Variable number of tandem repeat (VNTR) markers for human gene mapping. *Science* 235:1616–1622.
23. Atkinson K, MM Horowitz, RP Gale, MB Lee, AA Rimm and MM Bortin. (1989). Consensus among bone marrow transplanters for diagnosis, grading and treatment of chronic graft-versus-host disease. Committee of the International Bone Marrow Transplant Registry. *Bone Marrow Transplant* 4:247–254.
24. Przepiorka D, D Weisdorf, P Martin, HG Klingemann, P Beatty, J Hows and ED Thomas. (1995). 1994 consensus conference on acute GVHD grading. *Bone Marrow Transplant* 15:825–828.
25. Kaplan EL and P Meier. (1958). Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 53:457–481.
26. Barker JN, A Scaradavou and CE Stevens. (2010). Combined effect of total nucleated cell dose and HLA match on transplantation outcome in 1061 cord blood recipients with hematologic malignancies. *Blood* 115:1843–1849.
27. Sanz J, JC Boluda, C Martin, M Gonzalez, C Ferra, D Serano, CD de Heredia, C Barrenetxea, AM Martinez, et al. (2012). Single-unit umbilical cord blood transplantation from unrelated donors in patients with hematological malignancy using busulfan, thiotepe, fludarabine and ATG as myeloablative conditioning regimen. *Bone Marrow Transplant* 47:1287–1293.
28. Page KM, L Zhang, A Mendizabal, S Wease, S Carter, T Gentry, AE Balber and J Kurtzberg. (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.
29. Gluckman E, V Rocha, A Boyer-Chammard, F Locatelli, W Arcese, R Pasquini, J Ortega, G Souillet, E Ferreira, et al. (1997). Outcome of cord-blood transplantation from related and unrelated donors. Eurocord Transplant Group and the European Blood and Marrow Transplantation Group. *N Engl J Med* 337:373–381.
30. Rubinstein P, C Carrier, A Scaradavou, J Kurtzberg, J Adamson, AR Migliaccio, RL Berkowitz, M Cabbad, NL Dobrila, et al. (1998). Outcomes among 562 recipients of placental-blood transplants from unrelated donors. *N Engl J Med* 339:1565–1577.

31. Wagner JE, JN Barker, TE DeFor, KS Baker, BR Blazar, C Eide, A Goldman, J Kersey, W Krivit, 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.
32. Laughlin MJ, J Barker, B Bambach, ON Koc, DA Rizzieri, JE Wagner, SL Gerson, HM Lazarus, M Cairo, et al. (2001). Hematopoietic engraftment and survival in adult recipients of umbilical-cord blood from unrelated donors. *N Engl J Med* 344:1815–1822.
33. Barker JN. (2007). Who should get cord blood transplants? *Biol Blood Marrow Transplant* 13:78–82.
34. Rosenstock J and B Zinman. (2007). Dipeptidyl peptidase-4 inhibitors and the management of type 2 diabetes mellitus. *Curr Opin Endocrinol Diabetes Obes* 14:98–107.
35. Rocha V, M Labopin, G Sanz, W Arcese, R Schwerdtfeger, A Bosi, N Jacobsen, T Ruutu, M de Lima, et al. (2004). Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. *N Engl J Med* 351:2276–2285.
36. Laughlin MJ, M Eapen, P Rubinstein, JE Wagner, MJ Zhang, RE Champlin, C Stevens, JN Barker, RP Gale, et al. (2004). Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. *N Engl J Med* 351:2265–2275.
37. Takahashi S, J Ooi, A Tomonari, T Konuma, N Tsukada, M Ooiwa-Monna, K Fukuno, M Uchiyama, K Takasugi, et al. (2007). Comparative single-institute analysis of cord blood transplantation from unrelated donors with bone marrow or peripheral blood stem-cell transplants from related donors in adult patients with hematologic malignancies after myeloablative conditioning regimen. *Blood* 109:1322–1330.
38. Eapen M, V Rocha, G Sanz, A Scaradavou, MJ Zhang, W Arcese, A Sirvent, RE Champlin, N Chao, et al., Center for International, R Marrow Transplant, E Acute Leukemia Working Party and C National Cord Blood Program of the New York Blood. (2010). Effect of graft source on unrelated donor haemopoietic stem-cell transplantation in adults with acute leukaemia: a retrospective analysis. *Lancet Oncol* 11:653–660.
39. Brunstein CG, JA Gutman, DJ Weisdorf, AE Woolfrey, TE Defor, TA Gooley, MR Verneris, FR Appelbaum, JE Wagner and C Delaney. (2010). Allogeneic hematopoietic cell transplantation for hematologic malignancy: relative risks and benefits of double umbilical cord blood. *Blood* 116:4693–4699.
40. Chow R, A Nademanee, J Rosenthal, C Karanes, TH Jaing, ML Graham, E Tsukahara, B Wang, D Gjertson, et al. (2007). Analysis of hematopoietic cell transplants using plasma-depleted cord blood products that are not red blood cell reduced. *Biol Blood Marrow Transplant* 13:1346–1357.
41. Rocha V and HE Broxmeyer. (2010). New approaches for improving engraftment after cord blood transplantation. *Biol Blood Marrow Transplant* 16:S126–S132.
42. Kindwall-Keller TL, Y Hegerfeldt, HJ Meyerson, S Margevicius, P Fu, W van Heeckeren, HM Lazarus, BW Cooper, SL Gerson, et al. (2012). Prospective study of one- vs two-unit umbilical cord blood transplantation following reduced intensity conditioning in adults with hematological malignancies. *Bone Marrow Transplant* 47:924–933.
43. Frasson F, F Gualandi, M Podesta, AM Raiola, A Ibatici, G Piaggio, M Sessarego, N Sessarego, M Gobbi, et al. (2008). Direct intrabone transplant of unrelated cord-blood cells in acute leukaemia: a phase I/II study. *Lancet Oncol* 9:831–839.
44. Robinson SN, PJ Simmons, MW Thomas, N Brouard, JA Javni, S Trilok, JS Shim, H Yang, D Steiner, et al. (2012). *Ex vivo* fucosylation improves human cord blood engraftment in NOD-SCID IL-2Rgamma(null) mice. *Exp Hematol* 40:445–456.
45. Hoggatt J, P Singh, J Sampath and LM Pelus. (2009). Prostaglandin E2 enhances hematopoietic stem cell homing, survival, and proliferation. *Blood* 113:5444–5455.
46. Goessling W, RS Allen, X Guan, P Jin, N Uchida, M Dovey, JM Harris, ME Metzger, AC Bonifacino, et al. (2011). Prostaglandin E2 enhances human cord blood stem cell xenotransplants and shows long-term safety in preclinical nonhuman primate transplant models. *Cell Stem Cell* 8:445–458.

Address correspondence to:

Dr. Sherif S. Farag
 Division of Hematology-Oncology
 Department of Medicine
 Indiana University School of Medicine
 Walther Hall, R3-C414
 980 West Walnut Street
 Indianapolis, IN 46202

E-mail: ssfarag@iupui.edu

Received for publication November 12, 2012

Accepted after revision December 7, 2012

Prepublished on Liebert Instant Online December 27, 2012