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Characterization and Function of Cryopreserved Bone Marrow from Deceased Organ Donors: A Potential Viable Alternative Graft Source

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Abstract

Despite the readily available graft sources for allogeneic hematopoietic cell transplantation (alloHCT), a significant unmet need remains in the timely provision of suitable unrelated donor grafts. This shortage is related to the rarity of certain HLA alleles in the donor pool, nonclearance of donors owing to infectious disease or general health status, and prolonged graft procurement and processing times. An alternative hematopoietic progenitor cell (HPC) graft source obtained from the vertebral bodies (VBs) of deceased organ donors could alleviate many of the obstacles associated with using grafts from healthy living donors or umbilical cord blood (UCB). Deceased

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SUPPLEMENTARY MATERIALS

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organ donor-derived bone marrow (BM) can be preemptively screened, cryogenically banked for on-demand use, and made available in adequate cell doses for HCT. We have developed a good manufacturing practice (GMP)-compliant process to recover and cryogenically bank VB-derived HPCs from deceased organ donor (OD) BM. Here we present results from an analysis of HPCs from BM obtained from 250 deceased donors to identify any substantial difference in composition or quality compared with HPCs from BM aspirated from the iliac crests of healthy living donors. BM from deceased donor VBs was processed in a central GMP facility and packaged for cryopreservation in 5% DMSO/2.5% human serum albumin. BM aspirated from living donor iliac crests was obtained and used for comparison. A portion of each specimen was analyzed before and after cryopreservation by flow cytometry and colony-forming unit potential. Bone marrow chimerism potential was assessed in irradiated immunocompromised NSG mice. Analysis of variance with Bonferroni correction for multiple comparisons was used to determine how cryopreservation affects BM cells and to evaluate indicators of successful engraftment of BM cells into irradiated murine models. The *t* test (with 95% confidence intervals [CIs]) was used to compare cells from deceased donors and living donors. A final dataset of complete clinical and matched laboratory data from 226 cryopreserved samples was used in linear regressions to predict outcomes of BM HPC processing. When compared before and after cryopreservation, OD-derived BM HPCs were found to be stable, with $CD34^+$ cells maintaining high viability and function after thawing. The yield from a single donor is sufficient for transplantation of an average of 1.6 patients (range, 1.2 to 7.5). $CD34^+$ cells from OD-derived HPCs from BM productively engrafted sublethally irradiated immunocompromised mouse BM (>44% and >67% chimerism at 8 and 16 weeks, respectively). Flow cytometry and secondary transplantation confirmed that OD HPCs from BM is composed of long-term engrafting $CD34^+CD38^-CD45RA^-CD90^+CD49f^+$ HSCs. Linear regression identified no meaningful predictive associations between selected donor-related characteristics and OD BM HPC quality or yield. Collectively, these data demonstrate that cryopreserved BM HPCs from deceased organ donors is potent and functionally equivalent to living donor BM HPCs and is a viable on-demand graft source for clinical HCT. Prospective clinical trials will soon commence in collaboration with the Center for International Blood and Marrow Research to assess the feasibility, safety, and efficacy of Ossium HPCs from BM (ClinicalTrials.gov identifier NCT05068401).

INTRODUCTION

Allogeneic hematopoietic cell transplantation (HCT) is a lifesaving treatment for many malignant and nonmalignant hematologic diseases. According to the Center for International Blood & Marrow Transplant Research (CIBMTR), approximately 26,000 HCTs were performed in the United States in 2019 [1]. Nearly 7000 of these HCTs used allogeneic unrelated donor (URD) hematopoietic progenitor cell (HPC) grafts, consisting of HLA-typed aspirated whole BM (HPC, marrow) or mobilized peripheral blood stem cells (HPC, apheresis) from healthy volunteers. Cryopreserved umbilical cord blood (HPC, cord) is also an acceptable HLA-matched or HLA-mismatched donor source. The use of post-transplantation cyclophosphamide (PTCy) as graft-versus-host disease (GVHD) prophylaxis recently has been shown to enable crossing of the HLA barrier, allowing the successful transplantation of HLA-mismatched HPC, marrow or HPC, apheresis from haploidentical donors and mismatched unrelated donors (MMUDs) [2,3].

Yet, despite these diverse graft sources, an unmet need remains, particularly for ethnically diverse patient candidates for allogeneic HCT with underrepresented HLA alleles in donor registries [4]. Alternative graft sources within the available pool of URD volunteers, such as cryopreserved umbilical cord blood (UCB) and related haploidentical donors, potentially could overcome existing disparities in HLA genotypes; however, these sources are limited by inadequate cell dosages obtainable from UCB, a lack of haploidentical donor availability and suitability, and recipient sensitization to related donors. The use of URD grafts also is limited by the time required to find a suitable donor and to collect and process the graft; for example, the median time from initiating a formal search of the National Marrow Donor Program (NMDP), Be the Match Registry to collection and shipment of an URD graft is 87 days [5]. Disruptions of the supply chain can cause even longer delays, as generally was seen following the Coronavirus disease 2019 (COVID-19) pandemic. Such delays in performing allogeneic HCT can be associated with inferior patient outcomes, especially in patients with advanced disease or elderly patients with leukemia, who typically have short complete remission (CR) windows [6–8]. In addition, delays in HCT may require multiple exposures of chemotherapy or immunotherapy, potentially leading to emergent or enhanced organ toxicity, which could compromise a patient's candidacy for allogeneic HCT.

Cryogenically banked BM recovered from deceased (ie, brain dead) organ donors is a promising new graft source that could complement living donor and UCB registries, provide adequate cell doses, and reduce the time to transplantation. More than 60 years ago, pioneers in the HCT field recognized that deceased donors were a potential abundant source of therapeutic BM [9], and since then deceased donor BM has been used safely with minimal conditioning in >700 cases to promote immune tolerance [10–22]. Recovery of highly functional BM from deceased organ donors is conceptually similar to the procurement of organs and tissues by organ procurement organizations (OPOs), which have functioned successfully for decades. Last year, >40,000 organ transplantations and >1 million tissue transplantations were performed in the United States alone (data obtained from the unos.org website).

High-functioning BM cells that satisfy hematopoietic stem and progenitor cell (HSPC) quality acceptance concerns can be recovered from deceased donor vertebrae after procurement and cross-country shipping from geographically dispersed OPOs [23]. The current study advances the field by exploring 4 objectives: (1) to determine whether differences, possibly clinically meaningful, exist between BM cells recovered from living donors versus those obtained from deceased organ donors; (2) to determine whether the quality of deceased organ donor (OD) HPCs from BM is impacted by cryopreservation; (3) to determine whether OD BM HPCs engraft successfully in a sublethally irradiated murine model; and (4) to investigate whether any donor-related demographic or clinical characteristics are sufficiently predictive of OD BM HPC quality and yield, to be used prospectively as criteria for donor selection. Collectively, our results demonstrate that cryopreserved OD BM HPCs are comparable in content and functionally equivalent to HPCs from living donor BM, is not influenced by donor-specific clinical characteristics, and has the potential to serve as an alternative on-demand source for clinical HCT.

METHODS

Vertebrae Recovery and Manufacture of OD HPC, Marrow

During 2020 to 2021, vertebral bodies (VBs) from 369 consented and disease-screened brain-dead ODs were received for recovery and cryopreservation of HPC, marrow. After obtaining informed consent from family members, donor medical records and serology results were screened. Only donors consented for research and training purposes were used in the collection of research data beyond the data normally used in clinical testing of OD HPC, marrow. Donor inclusion criteria were (1) confirmed brain death, (2) age between 7 and 55 years, (3) nonsepticemic, and (4) confirmed disease- and pathogen-free. Relevant donor characteristics are presented in Table 1.

BM samples were recovered from deceased OD VBs in a centralized processing facility (Ossium Health, Indianapolis, IN), which has developed a good manufacturing practices (GMP)-compliant process for recovering, testing, and preserving BM cells. Except where noted, BM recovery and cryopreservation to produce Ossium HPC, marrow were carried out as described previously [23].

A portion of the VBs were used for process improvement and training, leaving 250 fresh samples for evaluation, of which 226 were also cryopreserved. The VB segments (minimum T8 to L5) recovered by OPOs were assigned unique identifiers and shipped under hypothermic conditions to the central GMP facility, where they were cleaned, cut, and ground. BM was eluted and filtered using BM filtration sets (Fresenius Kabi, Lake Zurich, IL), and a total nucleated cell (TNC) count was obtained using a Sysmex laser counter (Sysmex America, Lincolnshire, IL) to adjust to a standardized cell count. DMSO (OriGen Biomedical, Austin, TX) was added slowly to a final concentration of 10%.

Ossium OD HPC, marrow aliquots of 65 to 70 mL are packaged in 250-mL bags (OriGen Biomedical), and residual cells are packaged in 5-mL screw cap cryovials (Corning; Radnor, PA). Bags and vials were passively cooled to −86 ° C and transferred to vapor-phase liquid nitrogen storage, as described previously [24]. Live donor aspirated BM from 3 healthy volunteers was purchased from Lonza (Walkersville, MD) for comparative analysis.

In-Process and Release Testing Methods

Standard characterization of OD HPC, marrow was performed on concentrated BM prior to the addition of DMSO and following thawing after cryopreservation. Routine testing procedures have been described previously [23]. Flow cytometry was performed using a NovoCyte 2060R flow cytometer (ACEA Biosciences, San Diego, CA) equipped with 488 nm and 640 nm lasers, following the International Society of Hematotherapy and Graft Engineering guidelines to enumerate $CD45^+$ and $CD34^+$ cells [25]. All antibodies, conjugates, and stains used in this study are listed in Supplementary Table S1. Colony-forming unit (CFU) assays were performed using MethOcult Optimum medium (Stem Cell Technologies, Vancouver, BC, Canada).

Expanded Analyses

A subset of research-consented donor-derived OD HPC, marrow was characterized more extensively for lymphocyte subsets. Antibody combinations used to define specific lymphocyte subsets are listed in Supplementary Table S2.

Analysis of HSPC populations was performed on immunomagnetically selected $CD34^+$cells (CD34 isolation kit 17856; Stem Cell Technology) obtained from OD HPC, marrow or live donor iliac crest-aspirated BM obtained through Lonza (Walkersville, MD). HSC were defined as $CD34^+CD45RA^-CD38^-CD90^+CD49f^+$.

Irradiated NSG Mouse Engraftment

OD HPC, marrow was incorporated into a murine engraftment study, approved by the Indiana University School of Medicine Institutional Animal Care and Use Committee (approval no. 11394). The study was conducted using NOD.Cg-Prkdc^{scid} IL2r$_\gamma{}^{tm1Wjl}$/Sz (NSG) mice purchased from Jackson Laboratory (Bar Harbor, ME) [26]. Twenty 8- to 10-week-old NSG mice were sublethally irradiated with 300 cGy total body irradiation using a MARK I Model 68A Cs irradiator (JL Shepherd & Associates, San Fernando, CA) and then randomized in equal numbers to receive $CD34^+$ cells immunomagnetically selected from OD HPC, marrow (5×10^5). Cells were injected through the tail vein of mice immobilized in Plexiglas restrainers.

At 8 weeks postinjection, mice were immobilized in Plexiglas restrainers, tails were anesthetized with ethyl chloride (Topical Anesthetic Skin Refrigerant; Gebauer, Cleveland, OH), and peripheral blood was collected by tail snips for analysis of chimerism. At 16 weeks postinfection, the mice were euthanized, and blood, BM, and spleens were collected. Femurs, tibias, and fibulas were dissected, and BM was harvested by flushing with Iscove's modified Dulbecco's medium (Thermo Fisher Scientific, Waltham, MA) with .5% bovine serum albumin (Thermo Fisher Scientific). Spleens were mechanically dissociated in PBS. Cell suspensions were filtered through a 40-μm nylon cell strainer and then treated with red cell lysis buffer. Cells were stained with allophycocyanin-conjugated anti-human CD45 alone or in combination with phycoerythrin PE-conjugated anti-human CD33 to detect total human cell engraftment ($CD45^+$) and human myeloid cells ($CD33^+CD14^+$). Identical aliquots were stained with a combination of allophycocyanin-conjugated anti-human CD34, anti-human CD38- fluorescein isothiocyanate, and anti-human CD19-phycoerythrin to detect lin-$CD45^+CD34^+CD38^-$ HSCs, lin-$CD45^+CD34^+CD38^+$ progenitors, and lin-$CD45^+CD34^+CD19^+$ B cell progenitors. All antibodies were obtained from BD Biosciences (San Jose, CA). Human engraftment was defined as the presence of >.5% human cells in the blood and BM.

To establish the long-term repopulating potential of $CD34^+$ cells derived from OD HPC, marrow, secondary transplantations were performed with BM collected from each of the 10 mice receiving OD HPC, marrow $CD34^+$ cells. Secondary mice were irradiated and infused as described above with 10×10^6 whole BM cells. At 16 weeks, they were euthanized and BM, peripheral blood, and spleens were collected and analyzed for the presence of human $CD45^+$ cells by flow cytometry.

Statistical Tests and Predictive Modeling

Mean with standard deviation or median with interquartile range (IQR) were used to summarize donor demographic, risk factor, comorbidity, and cause of death data. The *t* test (with 95% confidence interval [CI]) was used to compare cells from deceased donors (n = 4) and those obtained from living donors (n = 3). Analysis of variance was used to determine how cryopreservation affects BM cells and to evaluate indicators of successful engraftment of BM cells into irradiated murine models. The Bonferroni method was used to correct type I error rates for multiple comparisons. All statistical analyses were performed using Prism 9 (GraphPad Software, San Diego, CA).

For predictive modeling, donor-related clinical characteristics (demographic, risk factor, comorbidity, and cause of death data) were abstracted from the Uniform Donor Risk Assessment Interview (UDRAI) form and from hospital records provided by the originating OPOs. These data were then matched to laboratory results (cell yield and viability) from cryopreserved OD HPC, marrow samples obtained from the same donors. The final dataset contained complete clinical and matched laboratory data from 226 cryopreserved samples. These data were then used in linear regressions to predict HPC, marrow outcomes. The regression analyses had 2 objectives: to determine whether (1) any of the differences in donor characteristics are significantly associated with differences in the yield of viable HPC, marrow, and if so, (2) whether those differences (effect sizes) are large enough to be of practical concern—that is, to warrant their use as criteria for donor selection or exclusion from the donor pool. In separate regression equations, 16 donor-related variables were regressed onto 8 HPC, marrow outcome measures. Because variation in VB processing is known to influence the composition and yield of recovered cells [23], 4 processing variables —warm ischemia and cold ischemia times during VB shipment, total VB processing time after arrival, and number of VBs processed per donor—also were included as predictors in the regressions. These additional predictors were included as control variables to avoid confounding VB process-related effects with the effects of donor-related characteristics. Binary predictor variables (eg, donor sex: male, female) were dummy coded (0,1). Because numeric variables were measured on different scales (ie, age in years, body mass index [BMI] in kg/m^2, processing time in hours), they were rescaled by subtracting the mean and dividing by two standard deviations, which placed them on a common scale and made them roughly equivalent to binary variables for the purpose of comparisons [27]. Details of the coding and rescaling of predictor variables are provided in Supplementary Table S3.

RESULTS

Characterization of Deceased Donor Cohort and Donor-Derived OD HPC, Marrow

A cohort of 250 deceased donors with complete clinical and laboratory data were available for analysis. Donor characteristics are summarized in Table 1. The median donor age was 37 years (range, 10 to 55 years), and 39% were female. The racial breakdown for this cohort was 73% White, 11% Black, 12% Hispanic/Latino, 3% Asian, and 1% other. The average BMI was 29.1 ± 7.4. Recorded comorbidities included chronic obstructive pulmonary disease (12%), diabetes mellitus (12%), hypertension (8.3%), and coronary artery disease (6%).

All donors met the criteria for brain death, with the causes of death reported as anoxia (44%), cerebrovascular accident/stroke (26%), and head trauma (30%). Cardiopulmonary resuscitation was performed on 57% of the donors. Based on medical records and interviews with surviving family members, 51% of the donors were current or former smokers and 17% abused alcohol (using the Centers for Disease Control and Prevention definition of ≥15 drinks/week for males and ≥8 drinks/week for females).

Critical parameters during recovery, transport, and processing that could impact OD HPC, marrow quality were captured for each vertebral column processed (Table 1). The median warm ischemia time (ie, the period between asystole and recovery of a vertebral column) was 3.2 hours (IQR, 2.4 to 5.9 hours). The median cold ischemia time (ie, the period between placing the vertebral column on ice and the start of processing to recover BM) was 35.2 hours (IQR, 29.8 to 39.2 hours). A median of 5.9 hours (IQR, 5.3 to 6.4 hours) from the start of processing to cryopreservation of the final product was required to process a median of 9 (IQR, 8 to 11) VBs per donor.

Comparison of Living Donor and Deceased Donor Bone Marrow

Comparisons were made between cells from OD-VB-derived HPC, marrow and living donor iliac crest (LD-IC)-aspirated BM. Whole BM as well as immunomagnetically selected $CD34^+$ cells were analyzed. No statistically significant differences between the 2 sources were detected in the viability of whole-BM $CD45^+$ WBCs, $CD34^+$ HSPCs, or $CD3^+$ T lymphocytes (Figure 1A-D). The percentage of $CD34^+$ HSPCs in the WBCs of OD HPC, marrow of deceased donors also did not differ significantly from that observed in LD-IC BM (mean, 1.2 ± .27% versus .89 ± .24%). However, the mean percentage of total $CD3^+$ T cells was approximately 3-fold lower in HPC, marrow than in LD-IC BM (6.8 ± 2.3% versus 18.4 ± 3.9%) (Figure 1E). Although no detectable difference in GM-CFU potential was observed between the 2 sources (Figure 1F), total CFUs were significantly higher in OD HPC, marrow (Figure 1G).

The population of $CD34^+$ cells was analyzed in more detail after enriching from whole BM. Long-term repopulating HSCs and multipotent progenitor cells were identified by flow cytometry using the gating scheme shown in Figure 2A. No statistically significant differences between BM sources were detected in the frequency of either of these cell types (Figure 2B,C) or in the frequencies of CFU progenitor subsets (Figure 2D). Taken together, results demonstrate that OD HPC, marrow is similar to LD-IC-aspirated BM.

Comparison of Fresh versus Cryopreserved BM from Deceased Donors

Testing was performed in a Clinical Laboratory Improvement Amendments-certified laboratory (Ossium Health, Indianapolis, IN) on 250 fresh OD HPC, marrow samples recovered during processing and 226 auxiliary vials analyzed after cryopreservation and thawing. Precryopreservation and postcryopreservation data are presented in Figure 3, and mean values for numeric outcome variables are provided in Supplementary Table S2. The mean total $CD45^+$ WBC concentration prior to cryopreservation was $1.2 \pm 0.5 \times 10^8$/mL, which did not differ significantly from that measured following cryopreservation ($1.1 \pm .83 \times 10^8$) (Figure 3A). The concentration of viable $CD34^+$ cells in fresh samples

exhibited a slight but statistically significant decrease ($P < .01$) after cryopreservation (9.6 ± 4.8 × 10^5/mL versus 7.8 ± 6.5 × 10^5/mL in fresh and post-thaw samples, respectively) (Figure 3B) and composed a similar percentage of viable WBCs recovered post-thaw (.93 ± .38% in fresh samples versus .95 ± .63% in post-thaw samples) (Figure 3C). Total and viable $CD3^+$ T cell concentrations within the WBC population were significantly lower following cryopreservation (Figure 3D and Supplementary Table S2). Compared with the total nucleated cell population, each of $CD45^+$ WBC, $CD3^+$ T cell, and $CD34^+$ HSPC populations declined slightly following cryopreservation (Figure 3E and Supplementary Table S2). The relatively large decrease in total viable WBC count most likely can be accounted for by a loss of neutrophils, which have been shown to be extremely sensitive to freezing [28].

The potential number of $CD34^+$ cell doses obtainable from each donor was determined based on average yields (Figure 3F). Ossium OD HPC, marrow is packaged in units of 65 mL total volume at 140 × 10^6 TNCs/mL, based on prefreezing counts. As shown in Figure 3F, there is an average of 9.6 × 10^5 viable $CD34^+$ HSPCs/mL, which represents 6.3 × 10^7 viable $CD34^+$ HSPCs per unit of OD HPC, marrow. The total number of viable $CD34^+$ cells recoverable from a single donor is 2.3 × 10^8, which equates to 3.7 units. Based on an adult HCT patient weighing 70 kg and requiring a minimum aspirated BM equivalent $CD34^+$ dose of 2 × 10^6/kg, the yield of $CD34^+$ cells is sufficient to transplant an average of 1.6 ± 1.2 patients and up to a maximum of 7.5 patients (upper range). Recent improvements in yields have increased the average number of units per donor to 4.8 (unpublished data), which translates to 2.1 HCT grafts recoverable per donor. Further improvements in processing, now being implemented, are expected to increase the yield further to nearly 3 grafts recoverable per donor. These data demonstrate that Ossium HPC, marrow from deceased organ donors can provide an abundant supply of highly functional BM cells for on-demand use in HCT procedures.

In addition to the standard characterization performed on all specimens, a subset of 6 samples was analyzed for an expanded panel of lymphocyte markers (Figure 3G). With the exception of an increased frequency of gamma-delta T cells ($P < .001$), populations of T cells, B cells, and natural killer cells remained stable throughout cryopreservation and thawing. Cryopreserved OD HPC, marrow maintained its CFU potential after thawing, although the number of lineage-committed progenitor cells was lower (Figure 3H). These data demonstrate that OD HPC, marrow is not substantially altered by cryopreservation and thawing.

Engraftment of OD HPC, Marrow

Enriched $CD34^+$ cells from 2 deceased donors were evaluated for their ability to engraft in nonlethally irradiated immunocompromised NSG mice (10 mice per donor) (Figure 4). The degree of chimerism by selected $CD34^+$ cells isolated from HPC marrow was analyzed at 8 weeks and 16 weeks postinjection. Human chimerism in peripheral blood was detectable at 8 weeks (Figure 4A). At the terminal 16-week time point, human BM chimerism was >65% in mice infused with OD HPC, marrow donor sources (75.0 ± 13.9% human $CD45^+$ for donor 1 and 66.6 ± 25.5% human $CD45^+$ for donor 2) (Figure 4B). Human leukocyte

subsets in BM also were detected in mice engrafted with OD HPC, marrow $CD34^+$ cells (Figure 4A-E). Detection of $CD3^+$ T cells was not attempted, given the well characterized nonpermissive environment of this mouse strain toward human thymocyte development [29]. Human cell chimerism also was detected in peripheral blood (Figure 4G) and spleen (Figure 4H) at 16 weeks. The CFU potential of $CD34^+$ cells from each cell donor was evaluated prior to injection (Figure 4I) and then again in BM recovered from injected mice at 16 weeks (Figure 4J) and was found to be similar for the 2 sources.

BM collected from each of the mice engrafted with $CD34^+$ cells obtained from the 2 OD HPC, marrow donors was subsequently used in secondary transplantations into a new set of sublethally irradiated NSG mice. At 16 weeks, engraftment was detected in peripheral blood and BM, demonstrating the presence of long-term engrafting of HSCs in the original OD HPC, marrow specimens (Figure 4K,L). These results demonstrate that the long-term engraftment potential of HSPC in deceased donor BM is retained during recovery and processing VBs to produce OD HPC, marrow.

Contribution of Donor-Related Clinical Characteristics to the Prediction of BM Cell Results

Results of separate linear regressions on 8 outcome indicators measured in 226 cryopreserved OD HPC, marrow samples are provided in Supplementary Table S4. Details of the scheme used to code predictor variables are provided in Supplementary Table S3. All regression coefficients are interpretable as effect sizes; the larger a coefficient's absolute magnitude, the greater its relative influence in predicting the outcome.

Five of the 8 regressions—those predicting TNC, granulocyte macrophage colony-forming units, total and viable $CD45^+$, and total $CD34^+$ counts—did not reach statistical significance, meaning that their predicted values did not exceed chance expectation. Three of the regressions—those for $CD34^+$ viability ($P < .025$), total $CD3^+$ count per milliliter ($P < .001$), and $CD3^+$viability ($P = .025$)—were statistically significant and accounted for 13%, 15%, and 11% of the total variance, respectively (Figure 5 and Supplementary Table S4). Previous research has shown (and Supplementary Table S4 confirms) that variability in VB processing affects the quality and yield of HPC, marrow [23]. For this reason, we included processing variables as statistical controls in the regression equations to separate their influence from the influence of donor-related characteristics. Thus, the donor-related predictions reported here are not confounded by processing effects.

The regression equation predicting percent viability of the $CD34^+$ subpopulation of cryopreserved $CD45^+$ cells was statistically significant ($P < .025$) and explained 13% of the variation in $CD34^+$ viability. The average viability of these cells was 79.2% (intercept). With other variables fixed, Black donors added +3.9% ($P < .05$) to the average viability, whereas other (non-White) donors subtracted −8.1% ($P < .01$). Head trauma (compared to anoxia) as the cause of death, was associated with a +2.9% increase in $CD34^+$cell viability ($P < .01$). None of the other donor-related characteristics were predictive of $CD34^+$cell viability.

Regression equations predicting total ($P < .001$) and viable ($P = .025$) $CD3^+$ cells were statistically significant and explained 15% and 11% of the total variance, respectively. The predicted average $CD3^+$ cell count was 6.7×10^6/mL, and the predicted viability was

46.8%. Donor age was a significant contributor to both predicted outcomes. With other variables fixed, a 2 SD (ie, 24 years) increase in age was associated with a 1.2×10^6 increase in $CD3^+$ T cells/mL ($P = .05$) and a 6.6% increase in cell viability ($P < .01$). Note that the large 2 SD increase in donor age (24 years) is associated with only a small increase in $CD3^+$cell viability, indicating that donor age, although statistically significant, is not a substantive concern within the range evaluated. None of the other donor-related characteristics were predictive of $CD3^+$cell viability. For $CD3^+$ cell count/mL, donor sex and BMI were statistically significant. Male donors were associated with a significant decrease in total $CD3^+$ of -1.3×10^6 ($P < .05$), and a 2 SD increase in BMI (+3.7 kg/m^2) was associated with a decrease of -1.5×10^6 $CD3^+$ cells/mL ($P < .01$). Interestingly, donors identified as having abused alcohol were associated with a 1.42×10^6/mL increase in total $CD3^+$ cell count.

These regression results show that most donor-related variables are not significantly associated with variation in BM cell composition or yield. The few individual coefficients that are statistically significant have numerical values that differ from 0 by an amount greater than would be expected to occur by chance, but their absolute magnitudes (effect sizes) are modest, meaning that they have little or no practical significance for use as donor selection criteria. Importantly, the relationships between BM cell composition and comorbid conditions, such as diabetes and hypertension, or the use of cardiopulmonary resuscitation prior to death were not statistically significant predictors.

DISCUSSION

Cryopreserved BM obtained from deceased organ donors could potentially address an unmet need as an alternative hematopoietic cell source. Our results show that OD HPC, marrow, has the following features: (1) it is comparable in composition and quality to BM obtained from living volunteers, (2) it is not substantially altered by cryopreservation, and (3) it contains functional HSPCs capable of engrafting and differentiating in a preclinical murine model, composed of long-term repopulating HSCs, as determined by secondary transplantation experiments. In addition, while regression analyses (Supplementary Table S4) revealed that some donor-related characteristics (ie, age, sex, race, BMI, and alcohol abuse) are predictably related to some HPC, marrow outcomes (ie, $CD3^+$ yield and $CD3^+$ and $CD34^+$ viability), their magnitudes (effect sizes) are not large enough to warrant their use as criteria for restricting donor selection, or to override the need to establish diverse graft sources for use in genetically heterogeneous recipient populations. These findings align with a recent study of 291 deceased organ donors that found very few differences in peripheral blood factors or leukocyte composition of deceased donors compared to living donors [30].

The use of deceased donor BM as a suitable clinical graft was suggested by a previous published case report of HCT with deceased matched related donor BM in the context of myeloablative conditioning to treat acute lymphoblastic leukemia [31]. Transplantation-related mortality was not observed in that study. Moreover, the clinical safety and functionality of deceased organ donor BM have been demonstrated in numerous studies designed to promote immune tolerance of transplanted solid organs and vascularized composite allografts [10,12–16,19,21,22,32]. The objective of these studies, which used

partial conditioning, was to promote operational tolerance through transient chimerism of donor VB-derived BM cells. Doses of $CD34^+$ cells in infused BM ranged from .3 to 3.4 × 10^6/kg (average 2.43 × 10^6/kg), which is in line with typical HCT doses. No deaths were reported in the combined >700 patients in these studies.

In the context of clinical HCT, the concentrations of both $CD34^+$ and $CD3^+$ cell populations are the 2 most important prognosticators for successful treatment of hematologic malignancies. Although a minimum dose of 2 × 10^6 BM $CD34^+$ cells/kg is generally considered necessary to prevent graft failure in the average HCT recipient, a sufficient range of T cells is also important to optimize beneficial graft-versus-tumor effects without promoting non-transplantation-related mortality from acute and chronic GVHD [33,34]. However, the optimal T cell concentration in allogeneic HCT grafts remains undefined [35]. A targeted Ossium OD HPC, marrow dose of 2 × 10^6 $CD34^+$ cells/kg contains on average 7.3 × 10^6 viable $CD3^+$ cells/kg, a T cell dose substantially lower than that in peripheral blood stem cell (PBSC) grafts. PBSC grafts account for approximately 80% of transplanted grafts and contain >11-fold more T cells than BM; however, they are associated with significantly higher chronic GVHD rates and the need for prolonged immunosuppressant therapy [36]. Thus, a lower T cell dose recovered in BM does not affect overall survival compared to PBSC grafts and may in fact contribute to beneficial outcomes, based on a composite GVHD, relapse-free survival (GRFS) endpoint, which incorporates quality of life metrics with nonrelapse mortality and overall survival [36]. Whether the composition of OD HPC, marrow is safe and efficacious for clinical use will depend on the results of pending clinical trials in the setting of HCT for hematologic malignancies.

One differentiating characteristic of OD HPC, marrow is its potential to be cryopreserved and banked for subsequent, on-demand use as a hematopoietic graft source. With the exception of UCB and autologous grafts, cryopreservation was not widely used for allo-HCT until very recently, when safety concerns regarding graft donation and logistical challenges with transport arose during the COVID-19 pandemic. Cryopreservation of grafts sometimes has been necessitated by disruptions in donation or patient readiness. These “emergency” cryopreservation cases have been analyzed retrospectively to compare HCT outcomes between fresh and post-thaw BM grafts [37–43]. Except for 1 single-center study [39], no clinically significant differences in the time to engraftment or overall survival have been reported between cryopreserved related donor and URD HCT for hematologic malignancies. A larger retrospective study conducted by the CIBMTR also found no differences in outcomes between combined related donor and URD HCT, for patients receiving cryopreserved BM grafts [44]. A second CIBMTR study that evaluated only patients treated prophylactically with PTCy also found no difference in outcomes between patients receiving fresh graft and those receiving cryopreserved grafts [45].

Limitations of the multicenter retrospective studies cited above were the frequent lack of clear records documenting cryopreservation and thawing protocols and the variability in cryopreservation and thawing practices between transplantation centers when records were available [37,46]. A single high-volume bank of cryopreserved URD grafts can better ensure standardization and consistency of storage parameters under optimized conditions. Standardized processing protocols overcome problems encountered with

emergency cryopreserved grafts, a significant number of which are not transplantable owing to deviations in procedures and packaging [37,46]. Protocols for cryopreserving and thawing Ossium-produced OD HPC, marrow have been optimized and validated [24], and detailed standard operating procedures for thawing and infusing the product are provided to transplantation centers. Combined with the centralized GMP production facility, these measures should reduce the variability among HCTs performed using this graft source.

These encouraging preclinical results must be corroborated in prospective clinical trials of HCT for hematologic syndromes following current standards of care. An initial phase 1 clinical safety trial is planned in collaboration with the CIBMTR, using MMUD Ossium-produced OD HPC, marrow with PTCy to treat hematologic malignancies (ClinicalTrials.gov identifier NCT05068401). Once evidence of clinical safety and efficacy is confirmed, OD HPC, marrow has the potential for rapid clinical adoption in HCT, using consistent evidence-based protocols and an established bank as an on-demand hematopoietic graft source that will be listed in the NMDP registry. Further studies could be designed to directly compare OD HPC, marrow to established alternative donor sources, such as haploidentical, MMUD, and UCB grafts.

A clear advantage of banked OD HPC, marrow over most other donor sources is its ready availability, which is especially important for addressing potential mass causality radiologic or nuclear incidents requiring HCT for many thousands of victims [47,48]. Scaling the bank to ensure adequate preparedness for large-scale nuclear or radiologic disasters could be accelerated through strategic investments in infrastructure and personnel to increase throughput of processing deceased OD VBs obtained from present clinically compliant OPOs, as well as training additional OPOs to ensure that essential quality attributes are maintained during the recovery of vertebral columns for shipment to Ossium for processing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Conflict of interest statement:

E.J.W. and B.H.J. are employed by and hold equity in Ossium Health, Inc; J.R.W. is a paid consultant of Ossium Health, Inc; W.S.G. is employed by Indiana University/IU Health and is consulting Medical Director for Ossium Health, Inc and for Cook Regentec; D.G., C.H.L., H.M.M., K.M.M. and A.M.S. are employees of Ossium Health, Inc; B.J.B., E.S., A.L.S., and K.E.P. are employees of the Indiana University School of Medicine; S.S. is an Employee of the National Marrow Donor Program (NMDP) and protocol team member for Ossium-sponsored PRESERVE I and PRESERVE II clinical trials. J.J.A. is an employee of the NMDP and protocol chair for the Ossium-sponsored PRESERVE I and PRESERVE II clinical trials.

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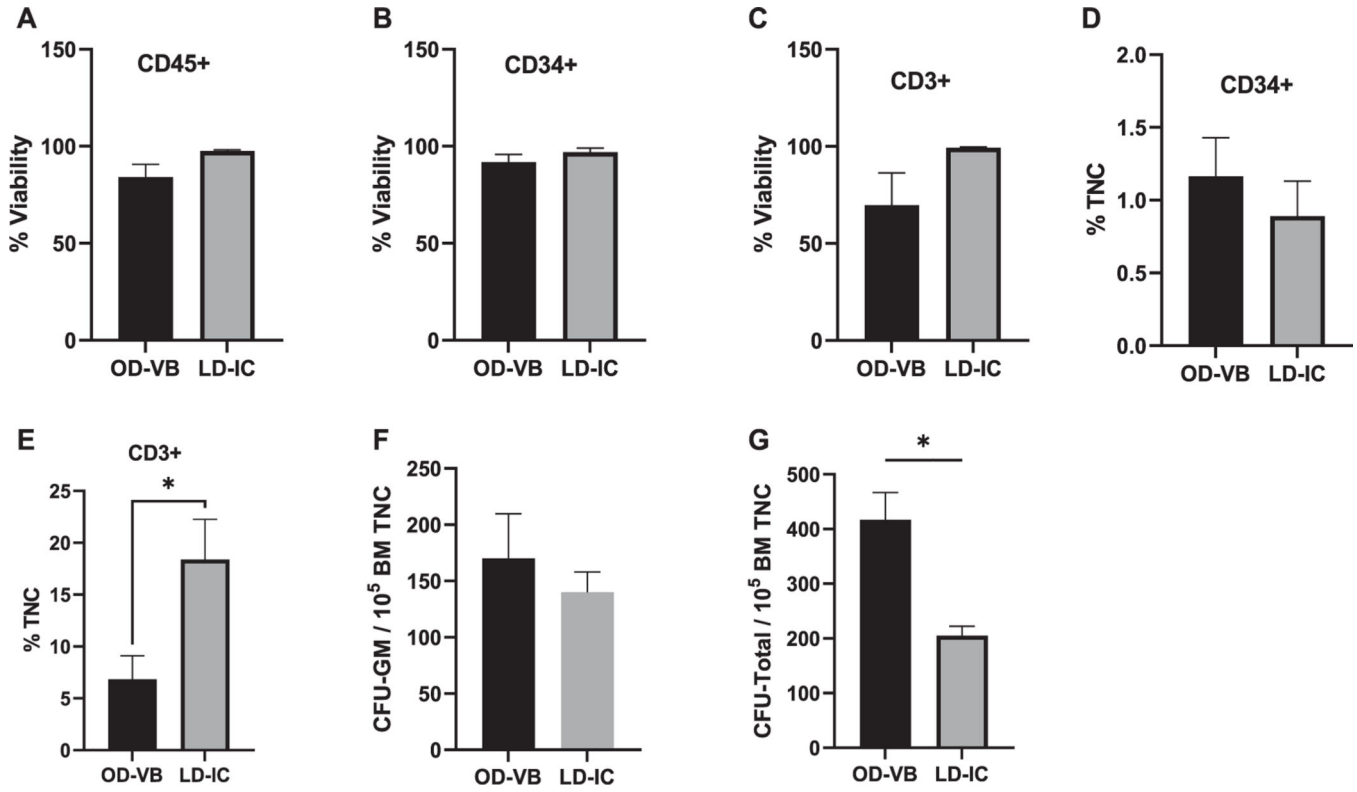


Figure 1.

Comparison of deceased OD VB-derived OD HPC, marrow and LD-IC-aspirated BM. (A) Viability of total $CD45^+$WBCs. (B) Viability of $CD34^+$HSPCs. (C) Viability of $CD3^+$ T cells. (D) Percentage of TNCs that are $CD34^+$ HSPCs. (E) Percentage of TNCs that are $CD3^+$ T cells. (F) Numbers of granulocyte macrophage colony-forming unit (CFU-GM) progenitors in 10^5 whole BM cells. (G) Numbers of total CFU progenitors in 10^5 whole BM cells. $*P < .05$, Welch 2-tailed *t* test.

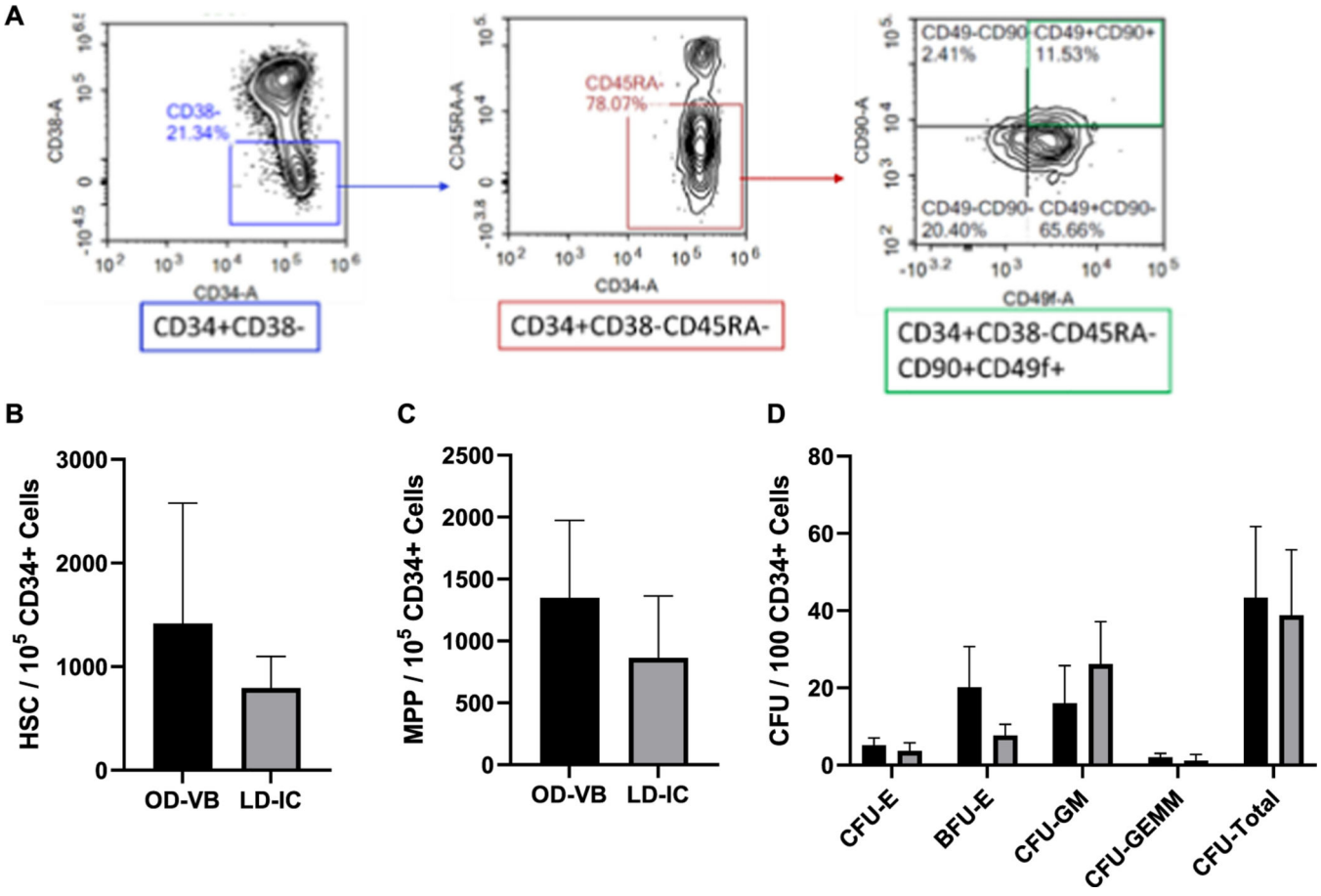
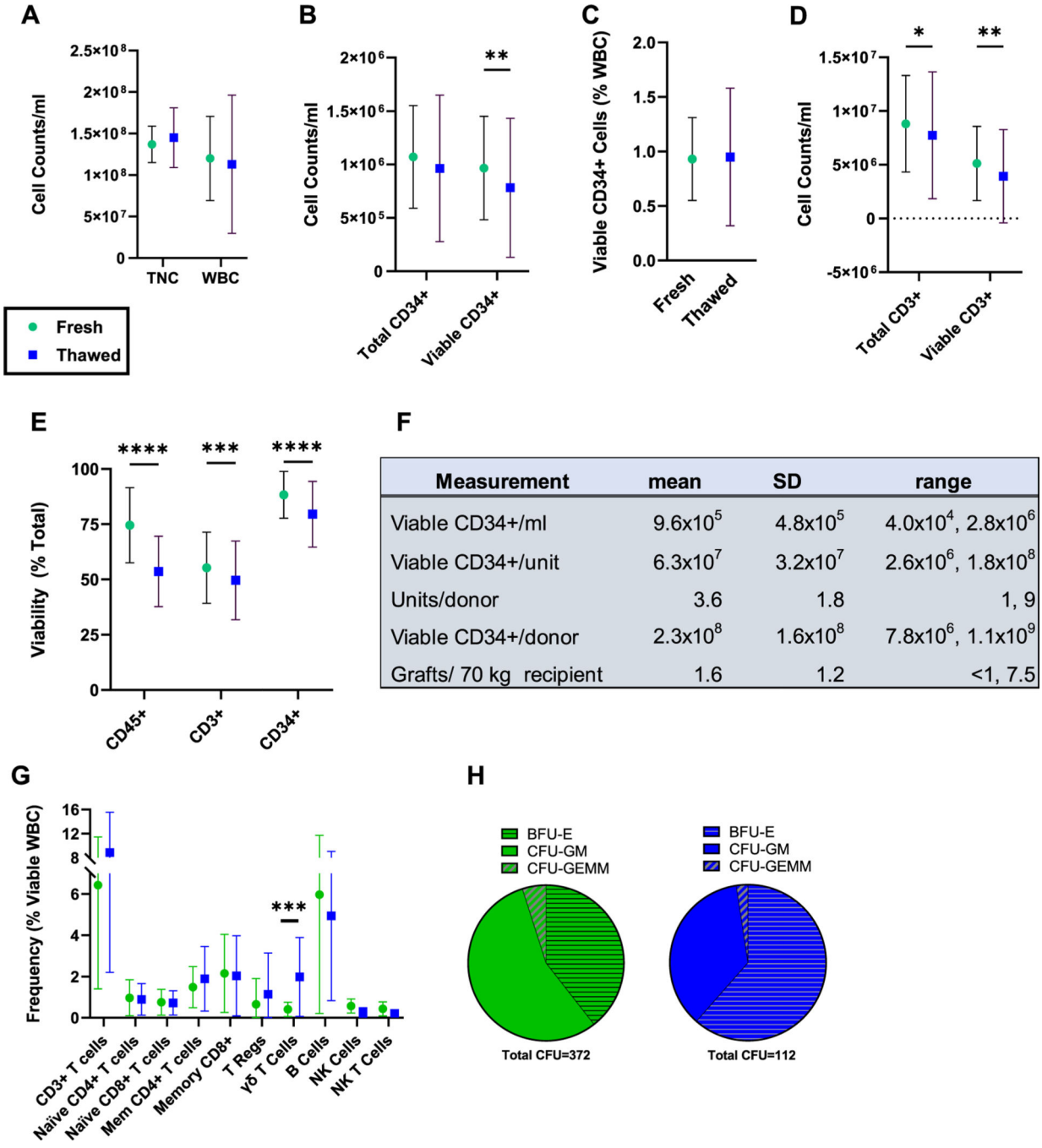


Figure 2.

Comparison of HSPC populations in the $CD34^+$ cell fraction of OD HPC, marrow and living donor BM. (A) Representative gating strategy to define and enumerate HSPCs. (B) Long-term repopulating HSC populations, defined as $CD34^+CD38^-CD45RA^-CD90^+CD49f^+$, in $CD34^+$ populations selected from OD-VB and LD-IC BM. (C) Multipotent progenitor (MPP) populations ($CD34^+CD38^+$) in $CD34^+$ populations selected from OD-VB and LD-IC BM. (D) CFU progenitor populations in selected $CD34^+$ cells from OD-VB (black bars) and LD-IC (gray bars) BM. CFU-E, CFU-erythroid; BFU-E, burst forming unit-erythroid; CFU-GEMM, CFU-granulocyte, erythroid, macrophage, megakaryocyte; CFU-total, the sum of individual progenitors.



Measurement	mean	SD	range
Viable CD34+/ml	$9.6x10^5$	$4.8x10^5$	$4.0x10^4$, $2.8x10^6$
Viable CD34+/unit	$6.3x10^7$	$3.2x10^7$	$2.6x10^6$, $1.8x10^8$
Units/donor	3.6	1.8	1, 9
Viable CD34+/donor	$2.3x10^8$	$1.6x10^8$	$7.8x10^6$, $1.1x10^9$
Grafts/ 70 kg recipient	1.6	1.2	<1, 7.5

Figure 3.

Characterization of fresh (green circles) and post-cryopreserved (blue boxes) OD HPC, marrow. (A) TNC and $CD45^+$WBC counts/mL. (B) Total and viable $CD34^+$ HSPCs/mL. (C) Viable $CD34^+$ cells as a percentage of WBCs. (D) Total and viable $CD3^+$ T cells/mL. (E) Viable percentages of $CD45^+$ WBCs, $CD3^+$ T cells, and $CD34^+$ HSPCs. (F) Mean ± SD and range $CD34^+$ HSPC counts. Viable $CD34^+$ cells/unit is the number of HSPCs in 65 mL stored in 250-mL blood bags. Units/donor is the number of bags obtained from a donor. Viable $CD34^+$ cells/donor is the total yield of HSPCs per donor. Grafts/70 kg patient

is the number of transplantations at 2×10^6 $CD34^+$ cells/kg that can be performed with the total CD34 cells yielded from each donor. (G) Frequency of viable WBCs positive for markers defining the indicated lymphocyte subsets. (H) Fractions of CFU subsets in fresh and cryopreserved HPC, marrow. The average total CFUs per 10^5 BM cells plated is indicated. $^*P < .05$; $^{**}P < .01$; $^{***}P < .001$; $^{****}P < 0.0001$, 2-way analysis of variance with Sidak's multiple comparison test. The total number of fresh samples was 250, of which 226 were analysed post-thaw. Six samples were analyzed in (G).

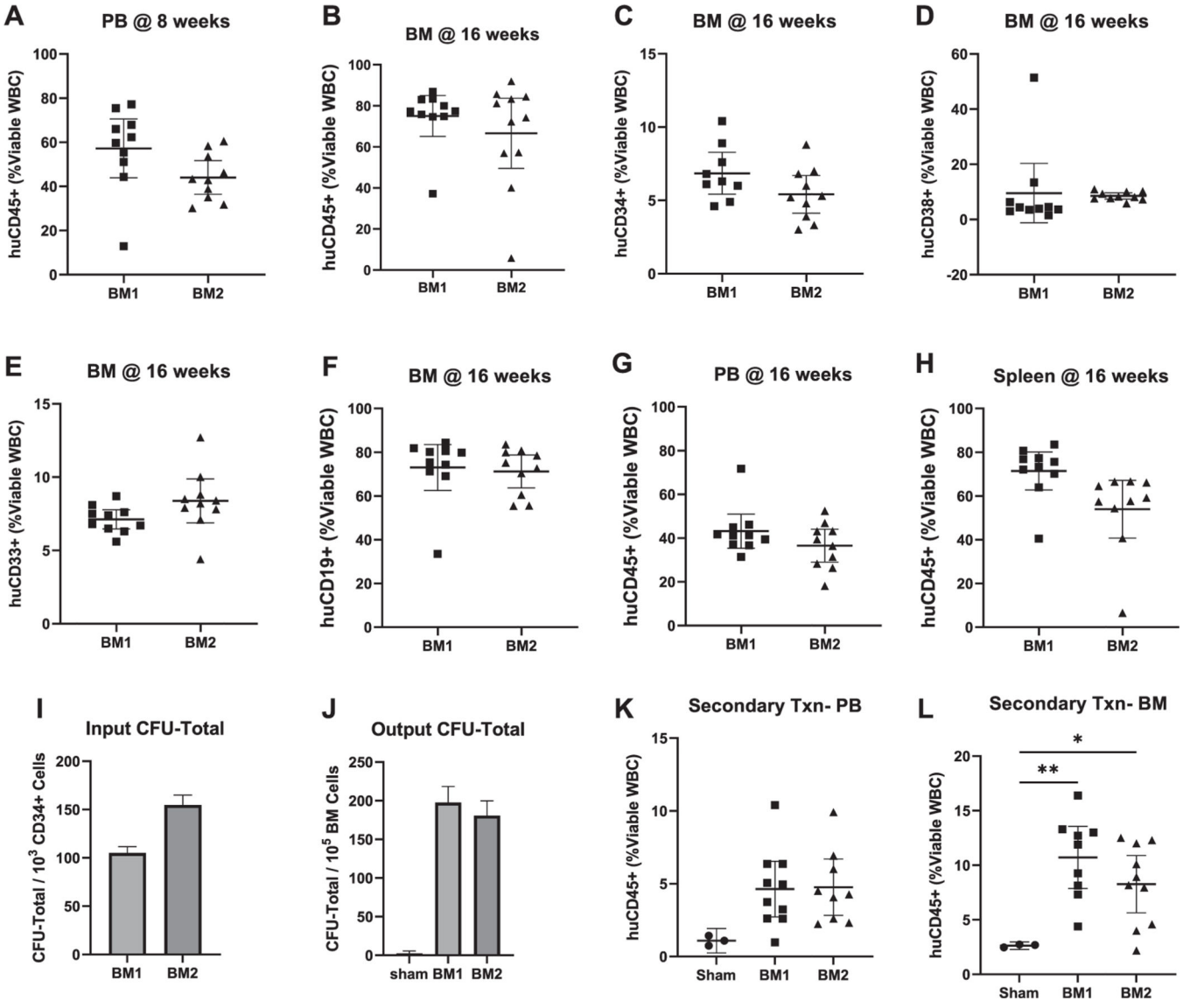


Figure 4.

Transplantation of cryopreserved human immunomagnetically selected $CD34^+$ cells from OD HPC, marrow recovered from 2 donors (BM1 and BM2). Immunocompromised NSG mice were irradiated at 300 cGy, followed by injection of $CD34^+$ cells at a dose of 5×10^5 through the tail vein. (A) Percentage of human $CD45^+$ cells in mouse peripheral blood at 8 weeks. (B-F)Analysis of BM at 16 weeks for percentage of cells expressing human surface epitopes for CD45 (B), CD34 (C), CD38 (D), CD33 (E), and CD19 (F). (G and H) Percentage of human $CD45^+$ cells in peripheral blood (PB) (G) and spleen (H) at 16 weeks. (I) Comparison of total CFU in cryopreserved selected $CD34^+$ cells. (J) Comparison of total human CFU in BM of mice at 16 weeks. (K and L) Secondary transplantations: human $CD45^+$ cells in peripheral blood (K) and BM (L) at 16 weeks following irradiation and injection with whole BM from mice receiving transplantation with HPC, marrow $CD34^+$ cells (10 NSG mice per group). $*P < .05$; $**P < .01$, $***P < .001$, analysis of variance with Tukey's multiple comparisons test.

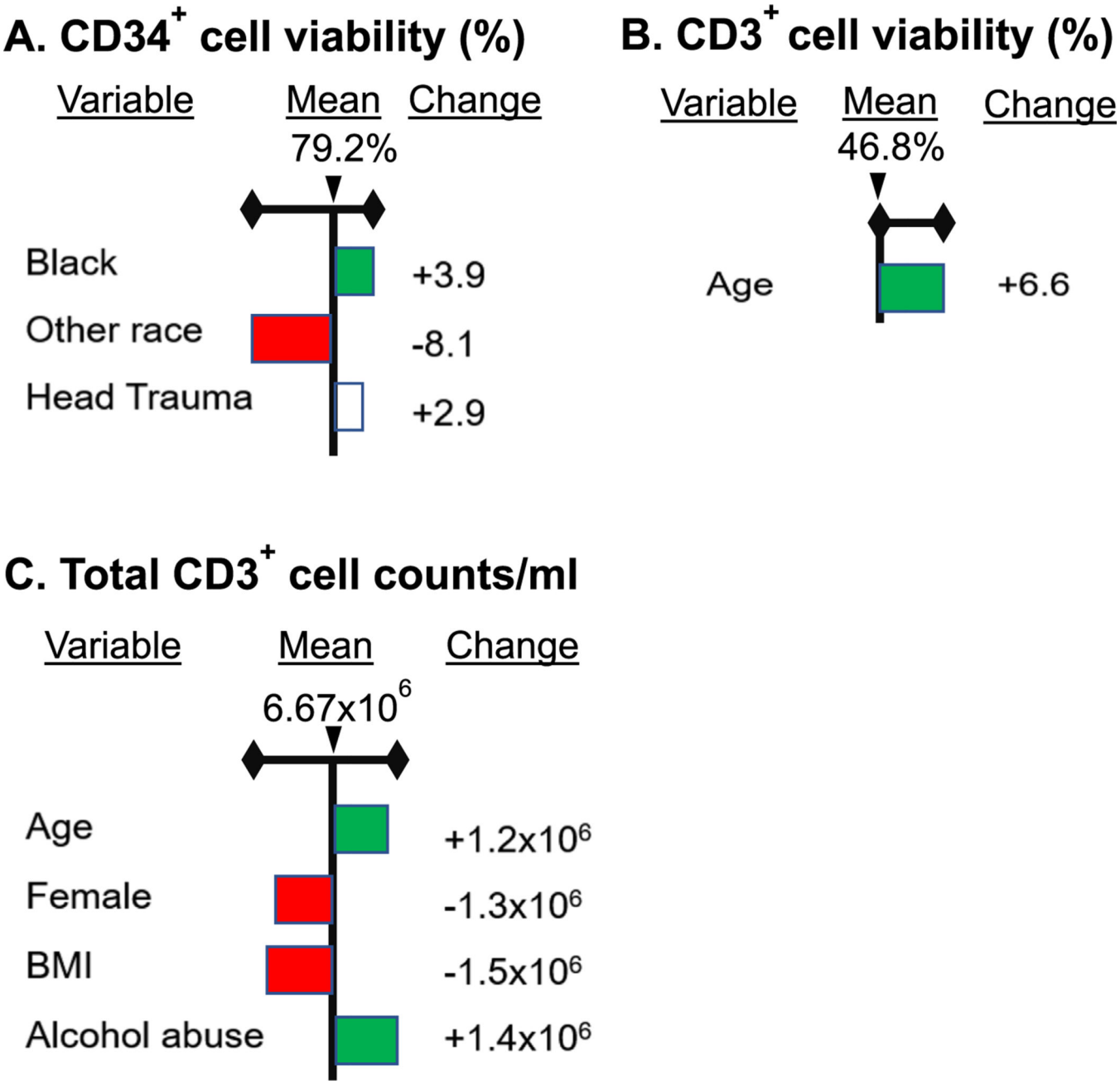


Figure 5.

Summary results from linear regression analyses showing the donor-related variables found to be significantly associated with variation in OD HPC, marrow composition. A complete list of the donor-related variables tested in the regressions is provided in Supplementary Table S4. Means (intercept values) are represented by vertical lines. The left columns identify the donor-related variables that were significantly associated with processed cell outcomes. The right columns show the values of regression coefficients associated with those donor-related variables. The bars indicate whether a donor-related variable had a negative (red) or positive (green) impact (ie, raised or lowered the slope, respectively) on the

outcome. The range is represented by the horizontal bar. Donor-related variables contributed significantly to OD HPC, marrow variation in $CD34^+$ cell viability (A), $CD3^+$ cell viability (B), and total $CD3^+$ cell count/mL (C).

Table 1

Donor Demographic, Medical History, and Circumstances of Death Characteristics

Characteristic	Value
Donor characteristics (N = 250)	
Sex, n (%)	
Female	87 (35)
Male	163 (65)
Race, n (%)	
White	183 (73)
Black	28 (11)
Hispanic/Latino	29 (12)
Asian	8 (3)
Other	3 (1)
Age, yr, median (IQR)	37 (25–45)
BMI, median (IQR)	29.1 (24.1–33.3)
At-risk behaviors, n (%)	
Current smoking	127 (51)
Alcohol abuse	43 (17)
Comorbidities, n (%)	
Chronic obstructive pulmonary disease/asthma	30 (12)
Diabetes mellitus	20 (8)
Hypertension	60 (24)
Coronary artery disease	15 (6)
Causes of death, n (%)	
Anoxia	110 (44)
Cerebrovascular accident/stroke	65 (26)
Head trauma	75 (30)
Clinical characteristics	
Cardiopulmonary resuscitation performed, n (%)	143 (57)
Recovery and processing details, median (IQR)	
Warm ischemia time, h	3.2 (2.5–5.9)
Cold ischemia time, h	35.2 (29.8–39.2)
VBs processed, n	9 (8–11)
Processing time, h	5.9 (5.3–6.4)