Biol Blood Marrow Transplant 23 (2017) 1463–1472

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

Manufacture of Autologous CD34+ Selected Grafts in the NIAID-Sponsored HALT-MS and SCOT Multicenter Clinical Trials for Autoimmune Diseases

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Article history:
Received 27 April 2017
Accepted 15 May 2017

Key Words:
Autologous hematopoietic cell transplantation
CD34 selection
Graft processing
Drug master file
Clinical trial
Multiple sclerosis
Systemic sclerosis (scleroderma)

A B S T R A C T

To ensure comparable grafts for autologous hematopoietic cell transplantation (HCT) in the National Institute of Allergy and Infectious Diseases–sponsored Investigational New Drug protocols for multiple sclerosis (HALT-MS) and systemic sclerosis (SCOT), a Drug Master File approach to control manufacture was implemented, including a common Master Production Batch Record and site-specific standard operating procedures with "Critical Elements." We assessed comparability of flow cytometry and controlled rate cryopreservation among sites and stability of cryopreserved grafts using hematopoietic progenitor cells (HPCs) from healthy donors. Hematopoietic Progenitor Cells, Apheresis-CD34+ Enriched, for Autologous Use (Auto-CD34+HPC) graft specifications included ≥70% viable CD34+ cells before cryopreservation. For the 2 protocols, 110 apheresis collections were performed; 121 lots of Auto-CD34+HPC were cryopreserved, and 107 of these (88.4%) met release criteria. Grafts were infused at a median of 25 days (range, 17 to 68) post-apheresis for HALT-MS (n = 24), and 25 days (range, 14 to 78) for SCOT (n = 33). Subjects received precryopreservation doses of a median 5.1 × 10^6 viable CD34+ cells/kg (range, 3.9 to 12.8) for HALT-MS and 5.6 × 10^6 viable CD34+ cells/kg (range, 2.6 to 10.2) for SCOT. Recovery of granulocytes occurred at a median of 11 days (range, 9 to 15) post-HCT for HALT-MS and 10 days (range, 8 to 12) for SCOT, independent of CD34+ cell dose. Subjects received their last platelet transfusion at a median of 9 days (range, 6 to 16) for HALT-MS and 8 days (range, 6 to 23) for SCOT; higher CD34+/kg doses were associated with faster platelet recovery. Stability testing of cryopreserved healthy donor CD34+ HPCs over 6 months of vapor phase liquid nitrogen storage demonstrated consistent 69% to 73% recovery of viable CD34+ cells. Manufacturing of Auto-CD34+HPC for the HALT-MS and SCOT protocols was comparable across all sites and supportive for timely recovery of granulocytes and platelets.

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INTRODUCTION

Clinical trials of autologous hematopoietic cell transplantation (HCT) for 2 autoimmune diseases, multiple sclerosis (MS) and scleroderma, opened during 2005, sponsored by the National Institute of Allergy and Infectious Diseases (NIAID). Clinical outcomes for the Phase II study in MS (HALT-MS) [1,2] and the prospective randomized study versus standard care for scleroderma (SCOT) [3] are available.

Even though processing for autologous CD34+ cell enrichment was regarded as minimal manipulation, the US Food and Drug Administration (FDA) determined use of this product for therapy of autoimmune diseases was nonhomologous and therefore subject to regulation under Section 351 of the Public Health Service Act. The protocols were conducted as 2 Investigational New Drug (IND) applications, and the manufacturing process for the autologous CD34+ selected graft was described in a Drug Master File (DMF), with the NIAID

Financial disclosure: See Acknowledgments on page 1471.

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http://dx.doi.org/10.1016/j.bbmt.2017.05.018
1083-8791/Published by Elsevier Inc. on behalf of the American Society for Blood and Marrow Transplantation.

serving as sponsor for the applications. The NIAID collaborated with experts in cell processing from the participating centers to develop the DMF for CD34 enrichment and cryopreservation. The objective was to ensure better control and standard methods of graft production, common to both clinical protocols at all clinical sites (Supplementary Appendix S2, Attachment I).

Here we describe our specifications for potency and purity for the cellular product, Auto-CD34$^+$HPC, and our proposals to the FDA for demonstration of comparability of manufacturing and analytical processes between the participating centers as well as for short-term stability of the final cryopreserved product. We correlated manufacturing data with engraftment outcomes to further assess our ability to meet the prespecified targets for safety, identity, purity, and potency of Auto-CD34$^+$HPC and the clinical efficacy of the grafts.

METHODS

Drug Master File

Specifications for potency and purity for Auto-CD34$^+$HPC

We specified viable CD34$^+$ HPC concentration measured after CD34$^+$ selection but before cryopreservation as our potency assay. Purity was defined as ≥70% total nucleated cell (TNC) viability and ≥70% of nucleated cells being viable CD34$^+$ cells in our type II DMF submission to the FDA for Auto-CD34$^+$HPC (BB-IND 11821; July 14, 2004; Supplementary Appendix S2, SOP 3000). To substantiate this choice, we provided data from the published literature in autologous HCT for malignancy, demonstrating faster neutrophil and platelet engraftment kinetics with higher CD34$^+$ cell content of bone marrow, mobilized peripheral blood, and CD34$^+$ selected products. CD34$^+$ cells/kg recipient body weight (RBW) ("dose") related to time to engraftment, with a threshold of 2 to 5×10^6/kg needed for recovery of granulocyte and platelet counts within about 14 days post-transplant (Supplementary Appendix S2, Attachment II). We also provided data from earlier Phase I/II studies in autoimmune diseases conducted at our participating sites. At CD34$^+$ cell doses ≥ 2×10^6/kg [4] for patients with MS, or ≥3.5×10^6/kg [5,6] for patients with MS or scleroderma, granulocyte counts recovered within about 10 days (data not shown).

Comparability of manufacturing processes at sites

Per the DMF, before participation manufacturing sites were qualified (Supplementary Appendix S2, Attachment III) by providing data for 3 batches of CD34-enriched cells produced using Baxter Isolex technology and performance of the Auto-CD34$^+$HPC potency assay. Facilities were required to be Current Good Manufacturing Practice compliant as confirmed by site inspection by a Good Manufacturing Practice expert.

Centers used site-specific standard operating procedures (SOPs) after NIAID review verified inclusion of "Critical Elements" of production described in common study-specific SOPs (Supplementary Appendix S2, Attachment IV). Comparability of product and process manufacture was ensured by implementation of the "Critical Elements" and by use of common Master Production (Supplementary Appendix S2, SOP 3001) and Master Postproduction (Supplementary Appendix S2, SOP 3002) Batch Records.

We conducted site-to-site comparability studies of flow cytometry techniques and controlled rate cryopreservation procedures. The usually discarded CD34$^+$ cell-depleted fraction remaining after CD34$^+$ selection, produced from mobilized peripheral blood of healthy volunteers at 1 site, the Fred Hutchinson Cancer Research Center (FHCRC), was used. To investigate comparability of flow cytometry techniques, samples of freshly processed CD34$^+$ cell-depleted fractions were shipped from FHCRC to the sites for measurement of TNC and flow cytometry analysis. Results from the sites were compared with a reference sample at FHCRC. To determine comparability of cryopreservation methods, each manufacturing site initiated controlled rate cryopreservation in Cryocyte bags with storage in the vapor phase of liquid nitrogen for ≥1 week; cryopreserved bags were then shipped back to FHCRC where after thaw and washing of samples to remove dimethyl sulfoxide (DMSO), measurement of TNC and analysis by flow cytometry was done, and results from other sites were compared with FHCRC.

Stability of Auto-CD34$^+$HPC with storage

We proposed Auto-CD34$^+$HPC would be infused within 3 months of the date of manufacture. Product was stored in the vapor phase of liquid nitrogen after controlled rate cryopreservation. We provided data to the FDA from published literature indicating grafts stored in this way supported engraftment after >10 years (Supplementary Appendix S2, Attachment V). Further, we provided data from prior Phase I/II studies in autoimmune diseases conducted at our participating sites [4-6] indicating products stored ≤ 1015 days supported robust engraftment (data not shown).

We conducted a 6-month stability study of controlled rate cryopreserved Auto-CD34$^+$HPC manufactured per the Master Production Batch Record using mobilized peripheral blood of a healthy volunteer at 1 site, FHCRC. A granulocyte colony-stimulating factor (G-CSF) mobilized healthy donor underwent 2 apheresis collections. These 2 collections were combined, and after CD34$^+$ selection enriched cells were aliquoted and cryopreserved with DMSO in 4 Cryocyte bags. One bag was thawed at 1 week and at 1, 3, and 6 months; after washing to remove DMSO, cell counts and flow cytometry were performed. Test parameters included potency (viable CD34$^+$ cells/mL), purity (proportions of viable TNC; viable CD34$^+$ cells), impurities (CD3$^+$ cells; other cells), appearance, and sterility. Product assessed before cryopreservation served as baseline for comparison of cell recovery and other assays at 1 week and later time points.

Manufacturing process control

Specifications for method(s) of apheresis collection of G-CSF mobilized peripheral blood stem cells (PBSCs) were per site-specific practice and were not regulated by our DMF. We defined the start of the manufacturing process for Auto-CD34$^+$HPC at the cell processing facility upon receipt of the apheresis product, which was considered the raw material. Process flow per the Master Production Batch Record (Supplementary Appendix S2, SOP 3001) provided for purification and characterization before cryopreservation and storage.

For CD34$^+$ enrichment, we used the Baxter Isolex (Baxter Healthcare Corporation, One Baxter Parkway, Deerfield, IL) 300i Magnetic Cell Selection System per the manufacturer's instructions, which specified a device load of ≤1000 mL containing ≤8×10^{10} TNC [7]. When production of Isolex was discontinued in 2010, we amended our DMF and the SCOT protocol, which had not yet completed enrollment, for substitution with the Miltenyi CliniMACS Instrument. Additional process flow specifications were per NIAID SOPs.

We defined a manufacturing "lot" as the product from 1 run of Isolex or CliniMACS CD34$^+$ cell selection, containing ≥3.5×10^6 viable cells. Depending on variables of the apheresis collection, processing of an individual subject's graft required manufacture of 1 or more lots of Auto-CD34$^+$HPC.

Product specifications and certificate of analysis

Auto-CD34$^+$HPC specifications included viable cells ≥ 70% TNC, viable CD34$^+$ ≥ 70% TNC, and impurities (including viable CD3$^+$ cells) ≤ 30% TNC (Supplementary Appendix S2, SOP 3000). Assessment by flow cytometry for CD34$^+$ and CD3$^+$ cells was performed before and after CD34$^+$ cell enrichment. Additional testing for CD56, CD19 or CD20, and CD14 was performed for further research characterization. Flow cytometry panels included 7-aminoactinomycin-D to assess overall viability. TNC and flow cytometry were used to determine absolute viable subset values (dual platform method) in the final CD34$^+$ cell enriched fraction. Sterility testing was performed after CD34$^+$ cell enrichment, on aliquots of each lot as described in 21CFR610.12. The Endotoxin assay (Endosafe, Charles River Laboratories, Wilmington, MA) was validated by each testing laboratory.

Clinical Protocols and Patients

Protocol ITN033AI (HALT-MS; BB-IND 12164; December 17, 2004; Clinicaltrials.gov NCT00288626) and Protocol SCSSc-01 (SCOT; BB-IND 11839; July 21, 2004; Clinicaltrials.gov NCT00114530) were approved by the institutional review boards at participating sites, and participants provided written informed consent. Participants self-identified race and ethnicity at screening. No financial compensation was provided. A total of 59 patients, 25 for HALT-MS and 34 for SCOT, were enrolled at 9 participating centers, 3 for HALT-MS and 8 for SCOT, from April 2006 to July 2011. Two subjects, 1 on each study, underwent graft manufacture and are included with the manufacturing data but did not proceed to transplant.

Mobilization of PBSCs

Subjects were mobilized for PBSC collection using G-CSF and prednisone, for the HALT-MS and SCOT studies, as described [1,2,6].

Graft Target Doses

The protocol-specified target dose of Auto-CD34$^+$HPC was ≥2×10^6/kg RBW for HALT-MS and ≥2.5×10^6/kg RBW for SCOT.

Engraftment Criteria

For HALT-MS, neutrophil engraftment was defined as an absolute neutrophil count > 500/μL for 2 consecutive measurements on different days. Platelet engraftment was defined as a platelet count > 20,000/μL for 2 consecutive measurements on different days, with no platelet transfusions in the preceding 7 days. For SCOT, engraftment was defined as achieving an absolute neutrophil count > 500 cells/μL and an unsupported platelet count > 20,000 cells/μL (unsupported defined as 7 days between last platelet transfusion and first of 3 consecutive daily platelet counts meeting this criterion).

These levels had to be maintained for 3 consecutive days. For both protocols, time to engraftment was defined as days elapsed between the day of graft infusion (day 0) and the day target cellularity was achieved.

Statistical Methods

Descriptive statistics were used to summarize attributes of the normal donor comparability and stability studies, apheresis collections, and final product composition. Data were summarized overall and by study, device, and site. No inferential analyses were conducted due to the exploratory and descriptive nature of this study. All figures were programmed using SAS version 9.4 (SAS Institute, Cary, NC).

RESULTS

Normal Donor Comparability Studies

Flow cytometry

TNC recovery was 70% to 103% in samples shipped to 8 sites versus 100% at the site of origin, FHCRC, at baseline (Figure 1). Viable CD34$^+$ cells were .06% to .12%, viable CD3$^+$ cells were 29% to 42%, and viable TNC were 77% to 99% of TNC at sites after shipment as compared with values of .13%, 37%, and 86%, respectively, at FHCRC at baseline. The observed differences seen in counts and cell subsets likely reflect random variations in aliquots as well as gating strategies, specific reagents used for staining, and instrument settings; none of those attributes was absolutely defined in the DMF.

Cryopreservation methods

TNC recovery for each site as compared with recovery for the reference center, FHCRC, was 62% to 106% post-thaw for samples shipped to the sites, cryopreserved, and returned to FHCRC for analysis (Figure 2). Viable CD34$^+$ cells were .11% to .20%, viable CD3$^+$ cells were 25% to 40%, and viable TNC were 71% to 80%.

Normal Donor Stability Study

There was an initial loss of 28% of viable CD34$^+$ cells at 1 week after the cryopreservation/thawing and washing process, but no further loss was associated with ongoing liquid nitrogen storage through 6 months (range, 27% to 31%) (Figure 3). Overall recovery of CD34$^+$ cells after thawing/washing was slightly better than CD3$^+$ cells. These results demonstrated ongoing stability and consistent recovery of about 70% Auto-CD34$^+$HPC for periods up to 6 months.

HALT-MS and SCOT study subjects received graft doses calculated based on characterization of Auto-CD34$^+$HPC performed before cryopreservation. Grafts were infused immediately post-thaw, without washing to remove DMSO or to reassess dose (Supplementary Appendix S2, SOP 3000 and 3001).

Mobilization of PBSCs

For HALT-MS, 24 of 25 subjects mobilized successfully with G-CSF and prednisone; 1 individual required subsequent mobilization using cyclophosphamide [1,2]. For SCOT, of 34 subjects treated with G-CSF and prednisone for mobilization, 2 needed a second course of G-CSF and prednisone, whereas a third required cyclophosphamide to achieve successful mobilization. No meaningful correlation of these events with clinical outcomes was observed.

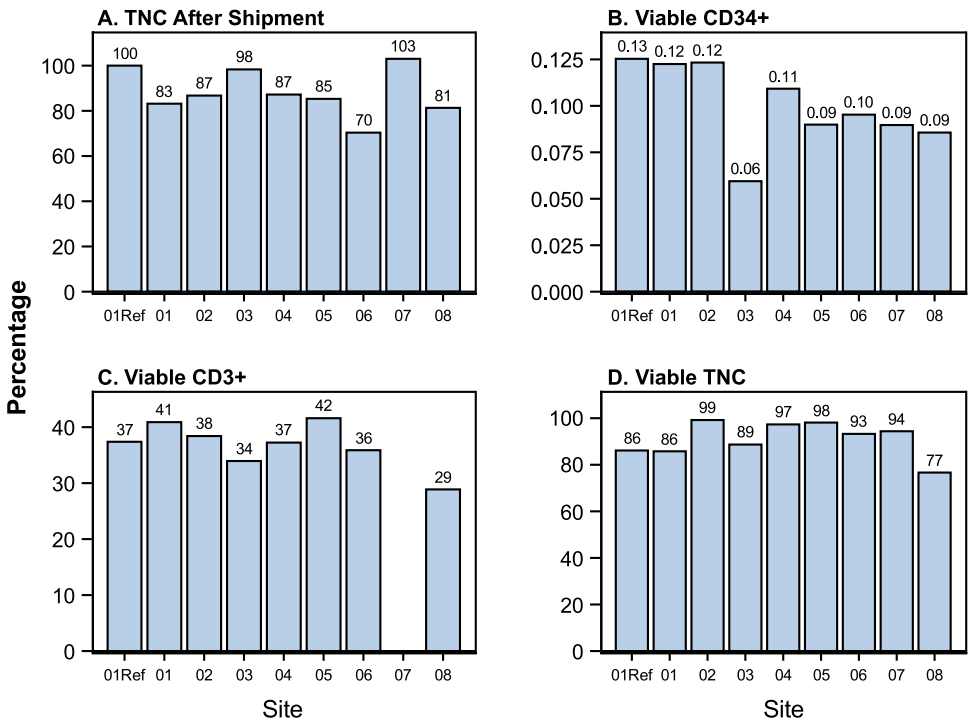

Figure 1. Comparability of flow cytometry techniques at sites. The CD34$^+$ cell depleted flow-through after Isolex, from a PBSC collection from a normal volunteer, was distributed into multiple aliquots in 15-mL centrifuge tubes (approximately 2 billion cells per sample, at a cell concentration of 1 to 2×10^8/mL). Site 01, where the samples originated, shipped tubes overnight for early morning receipt to 7 other sites and shipped a tube to itself to serve as an intralaboratory control (listed as 01). In addition to the 8 shipments, 1 study tube was retained in a refrigerator at the site of origin to serve as a control to assess effect of transport on product flow cytometry characteristics (listed as 01Ref). TNC measurement and flow cytometry analysis were performed at sites. (A) Percentage TNC Recovery was calculated by dividing the TNC result at each individual site by the TNC result for the reference tube stored at the site of origin (01Ref). (B-D) Percentages were calculated based on TNC (viable + nonviable) determined at the given site. In C, note that CD3$^+$ data at site 07 was not available. Site 01, FHCRC; Site 02, City of Hope; Site 03, Duke University; Site 04, Medical College of Wisconsin; Site 05, MD Anderson Cancer Center; Site 06, Ohio State University; Site 07, Dana Farber Cancer Institute; Site 08, University of Michigan.

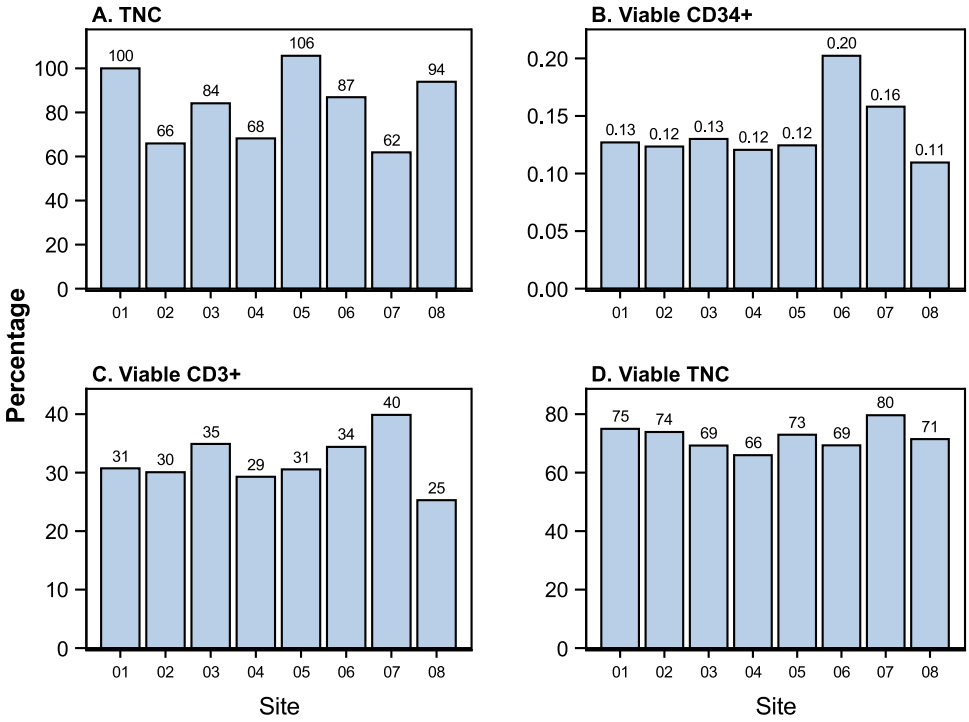

Figure 2. Comparability of controlled rate cryopreservation at sites. The CD34-depleted flow-through after Isolex, from a PBSC collection from a normal volunteer, was distributed into multiple aliquots in 15-mL centrifuge tubes and shipped from Site 01 to the other sites and to Site 01 as described in Figure 1. Sites then processed the samples for cryopreservation in Cryocyte bags. The Cryocyte bags were subjected to controlled rate freezing at the sites, stored for 1 week, and then shipped back to Site 01 where they were thawed and measured for TNC and analyzed by flow cytometry. (A) Percent TNC Recovery was calculated by dividing the TNC result from each individual site by the TNC result from the thawed product at Site 01. (B-D) Percentages were calculated using TNC determined at the given site. Sites are as in Figure 1.

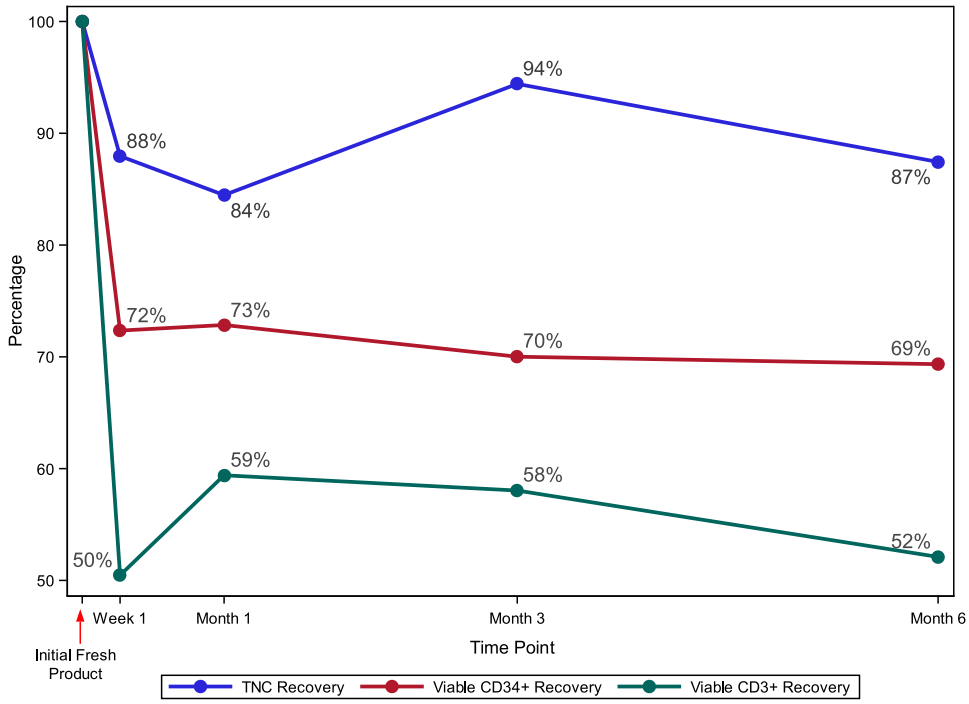

Figure 3. Stability of normal donor Auto-CD34⁺ HPC with storage. CD34⁺ cell enrichment using Isolex was performed on PBSCs from a G-CSF mobilized normal volunteer, and the enriched CD34⁺ cells were aliquoted into 4 Cryocyte bags and subjected to controlled rate cryopreservation. Bags were thawed at 1 week and at 1, 3, and 6 months and measured for TNC and analyzed by flow cytometry. TNC recovery was calculated by dividing TNC at the respective time point by TNC in the initial product before cryopreservation. Percentage viable CD34⁺ cell recovery was calculated by dividing number of viable CD34⁺ cells at the respective time point by the number of viable CD34⁺ cells in the initial product before cryopreservation. Percentage viable CD3⁺ recovery was calculated similarly.

Table 1
Manufacture and Disposition of Lots

A. Manufacture of lots from apheresis collections (n = 110)

	Isolex	CliniMACS
Single collection	84	8
Split* of 1 and/or pool of 2 collections	29	0
Total lots manufactured	113†	8‡

B. Disposition of lots

	Infused	Not Infused	Total
Met release criteria	105	2§	107
Did not meet release criteria	8¶	5‖	13
Unknown if met release criteria	0	1**	1
Total	113	8	121

C. Lots infused per subject††

Number of Lots Per Subject	HALT-MS, Number of Subjects (n = 24)	SCOT, Number of Subjects (n = 33)	Number of Lots Infused
1	4	13	17
2	15	16	62
3	4	3	21
4	1	0	4
9	0	1	9
Total			113

* Split of 1 apheresis collection to manufacture 2 lots of Auto-CD34⁺HPC.
† Manufactured for 25 subjects on HALT-MS, and 28 subjects on SCOT.
‡ Manufactured for 6 subjects on SCOT
§ One product for a SCOT subject was not infused because the subject was not transplanted. One product for a HALT-MS subject was not infused as the subject received another product (dose 6.8×10^6/kg) that exceeded the target dose for the protocol.
¶ Eight products that did not meet release criteria were infused as graft for 3 subjects. One SCOT subject received 5 failed products, 1 due to sterility criteria (presence of *Priopionibacterium*) and 4 due to purity criteria (range, 13% to 68% viable CD34⁺ cells). Two HALT-MS subjects received 3 products that did not meet purity criteria (65% and 63% viable CD34⁺ cells and 66% viable CD34⁺ cells).
‖ Products for 3 SCOT subjects (4 products) and 1 HALT-MS subject (1 product) were not infused because of failure of purity criteria for viable CD34⁺ cells.
** One product for a HALT-MS subject was not infused because the subject was not transplanted; it is unknown if the product met release criteria.
†† For HALT-MS, 4 subjects received 1 lot, 15 received 2 lots, 4 received 3 lots, and 1 received 4 lots of product. For SCOT, 13 subjects received 1 lot, 16 received 2 lots, 3 received 3 lots, and 1 received 9 lots of Auto-CD34⁺HPC.

Apheresis Collections

Characteristics of apheresis collections for HALT-MS and SCOT subjects were analyzed by site (Supplementary Appendix S1, Figure S1). A total of 110 apheresis collections were performed for the HALT-MS and SCOT protocols (Table 1A). These contained a median of 6.48×10^{10} TNC, of which a median of 3.77×10^8 were CD34⁺ cells and a median of 1.33×10^{10} were CD3⁺ cells; viability was a median of 99.1%. Apheresis collections were not regulated by the DMF. Furthermore, different individual subjects were collected at the different centers, so that subject factors and center-specific factors, including duration of the apheresis procedures and the instrument used, were likely important for these outcomes.

We compared the composition of apheresis collections between the HALT-MS and SCOT studies (Supplementary Appendix S1, Figure S2); no substantial differences for TNC, viable CD34⁺ cell, or viable CD3⁺ cell content were observed. A median of 3.6×10^8 viable CD34⁺ cells were present per apheresis collection for HALT-MS and a median of 3.9×10^8 CD34⁺ cells for SCOT.

Isolex and CliniMACS Device Function and Comparability

Purity of Auto-CD34⁺HPC lots manufactured using Isolex or CliniMACS and recovery of viable CD34⁺ cells from the apheresis collection loading material for the device were analyzed. For all centers and all lots, median viable CD34⁺ cell purity was 91% and median recovery of CD34⁺ cells was 59% (Supplementary Appendix S1, Figure S3). Variables with potential to influence these outcomes included subject-, center-, and device-specific factors.

We analyzed the cellular composition of Auto-CD34⁺HPC lots manufactured based on the clinical study, HALT-MS (Isolex only) or SCOT (Isolex and CliniMACS). No substantial differences were observed for CD34⁺ cell recovery, purity of CD34⁺ cells, log T cell depletion (TCD), or viability (Supplementary Appendix S1, Figure S3) for the 2 studies.

For the SCOT study, a single subject at Site 03 contributed to differences observed between this center and the others (Table 1B and Supplementary Appendix S1, Figure S3). During Isolex processing, samples for this individual repeatedly aggregated, causing cell loss. Despite the challenges in graft preparation for this subject, the individual engrafted successfully and recovered granulocyte and platelet counts in a timely manner.

We further analyzed the cellular composition of Auto-CD34⁺HPC lots based on the device used for CD34⁺ cell selection (Supplementary Appendix S1, Figure S4), including purity of Auto-CD34⁺HPC, viability, recovery of CD34⁺ cells from the apheresis collection, and log TCD. Products manufactured on CliniMACS had higher viable CD34⁺ cell purity and were more highly depleted of CD3⁺ cells than those processed on Isolex (Supplementary Appendix S1, Figure S4), consistent with historic data [8-11]. The median recovery of CD34⁺ cells from apheresis collections and the median viability for Auto-CD34⁺HPC were similar for the 2 devices. Although a total of 113 lots were processed on Isolex for subjects enrolled on HALT-MS or SCOT, only 8 lots were processed on CliniMACS for subjects enrolled on SCOT (Table 1B), limiting statistical comparison of the 2 groups.

For separations on Isolex, we analyzed the importance of the number of TNC, viable CD34⁺ cells, and viable CD3⁺ cells loaded on potency and purity of the resulting Auto-CD34⁺HPC (Supplementary Appendix S1, Figure S5). For viable CD34⁺ cell recovery, the strongest associations appeared to be TNC loaded and number of CD34⁺ cells loaded. However, neither TNC loaded nor number of CD3⁺ cells loaded affected the extent of CD3⁺ cell depletion. Utilization of the Isolex device as specified by the manufacturer resulted in a viable CD34⁺ cell enriched product with median recovery of 59% of the CD34⁺ cells loaded, with a median log TCD of 4 for viable CD3⁺.

Cell Composition of Grafts (Lots)

Product specifications for Auto-CD34⁺HPC (Supplementary Appendix S2, SOP 3000) were met or exceeded for 107 of 121 manufactured lots (88.4%) (Table 1B). We analyzed the cellular composition of manufactured lots based on the device used for purification and by study protocol (Figure 4). Median percentage of viable CD34⁺ cells constituted well above the 70% specification, with lots prepared on CliniMACS having a higher percentage on average as compared with Isolex (Figure 4A). Lots prepared on Isolex had a higher percentage of residual CD3⁺ cells on average, compared with CliniMACS (Figure 4B). The percentage of B cell, natural killer cell, and monocyte content was also analyzed (Figure 4C,D), with median values for B cell contamination being slightly higher for Isolex as compared with CliniMACS and median values for natural killer cell and monocyte contamination being similar for the devices. Final product viability met specifications for all lots with no more than 15% dead cells in lots

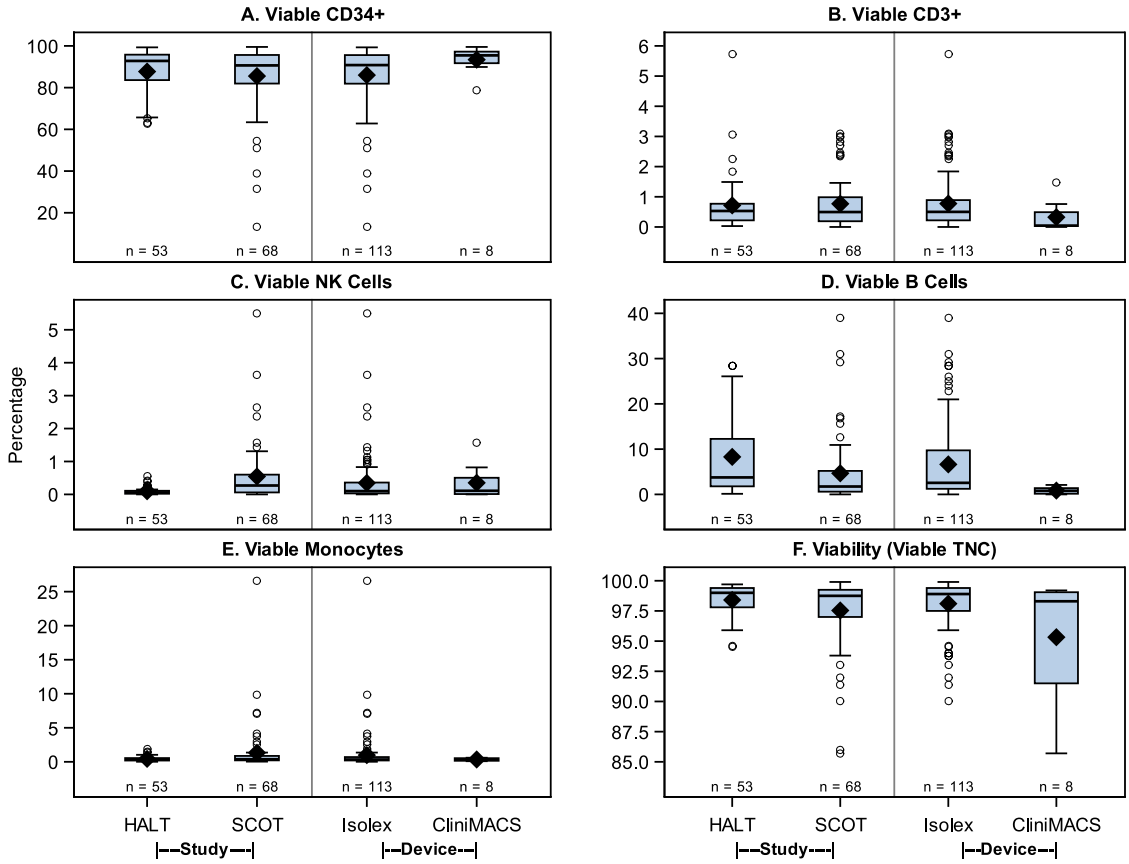

Figure 4. Auto-CD34⁺HPC composition by study and device. By Device: Median percentage of viable CD34⁺ cells constituted well above the 70% specification, with lots prepared on CliniMACS having a higher percentage on average as compared with Isolex (A). Lots prepared on Isolex had a higher percentage of residual CD3⁺ cells on average, compared with CliniMACS (B). Final product viability met specifications for all lots with no more than 15% dead cells in lots processed on either device (F). The percentage of B cells, natural killer cells, and monocyte content was also analyzed (C, D, and E). B cell (range, 0% to 2.1%) and monocyte (range, .1% to 6%) content in the lots processed on CliniMACS was uniformly low. B cell (range, 0% to 38.9%) and monocyte (range, .01% to 26.6%) contamination was greater for the lots processed on Isolex. B cell content for lots processed on Isolex was >10%, with 12 lots having B cell content > 20%. Of note, lots from a given patient tended to have similar levels of B cell contamination (data not shown). Although the median proportion of monocytes was the same for both devices (.34%), 5 lots processed with Isolex had ≥4% monocytes, with 1 as high as 26.6%. Lots from both devices had very low numbers of natural killer cells (<5.5%). By Study: The composition of lots prepared from patients on the HALT-MS and SCOT studies were very similar (A-F).

processed on either device (Figure 4F). The composition of lots prepared from patients on the HALT-MS and SCOT studies were very similar (Figure 4A-F).

Storage of Cryopreserved Grafts

Subjects received grafts that were CD34⁺ selected, cryopreserved, stored in the vapor phase of liquid nitrogen, thawed, and infused a median of 25 days (range, 17 to 68) after apheresis collection for HALT-MS and 25 days (range, 14 to 78) after apheresis collection for SCOT (Figure 5). The maximum duration of storage for the HALT-MS and SCOT studies, 68 days and 78 days, respectively, is within the 3-month period proposed by the NIAID to the FDA for stability of product and also within the 6 months' duration evaluated for stability using the healthy donor product for graft storage (Figure 3).

Graft Doses and Transplants

During May 2006 to August 2011, a total of 57 autologous transplants were performed, 24 for HALT-MS and 33 for SCOT (Table 1C and Figure 6A). Subjects received a median dose of 5.1×10^6 viable CD34⁺ cells/kg RBW (range, 3.9 to 12.8) for HALT-MS and 5.6×10^6 cells/kg RBW (range, 2.6 to 10.2) for SCOT. Auto-CD34⁺HPC doses met or exceeded the target doses for the protocols for all subjects. Subject grafts also included a median of 23.4×10^3 viable CD3⁺ cells/kg RBW (range, 5.1 to 213.5) for HALT-MS and 20.3×10^3 cells/kg RBW (range, 1.1 to 209.2) for SCOT (Figure 6B). As described above, for the apheresis collection as compared with the Auto-CD34⁺HPC product, this represents about 4 logs TCD using Isolex and 5 logs TCD with CliniMACS (Supplementary Appendix S1, Figure S4D). Neither CD34⁺ dose (Figure 6C) nor CD3⁺ content (Figure 6D) of the grafts received appeared to impact event-free survival for HALT-MS or SCOT.

Engraftment Kinetics

For both the HALT-MS and SCOT studies, all subjects successfully engrafted and recovered neutrophil and platelet counts in a timely manner. For SCOT, subjects achieved absolute neutrophil counts > 500/ μL at a median of 10 days post-HCT, whereas for HALT-MS, subjects achieved this at 11 days post-HCT (Figure 7A). SCOT subjects received their last platelet transfusion at a median of 8 days (range, 6 to 23) post-HCT, as compared with 9 days (range, 6 to 16) for HALT-MS (Figure 7B). Although days to recovery of neutrophils was not correlated with doses of Auto-CD34⁺HPC $>2 \times 10^6$/kg (Figure 7C), days to last platelet transfusion was somewhat sensitive to graft dose. Subjects receiving higher CD34⁺/kg

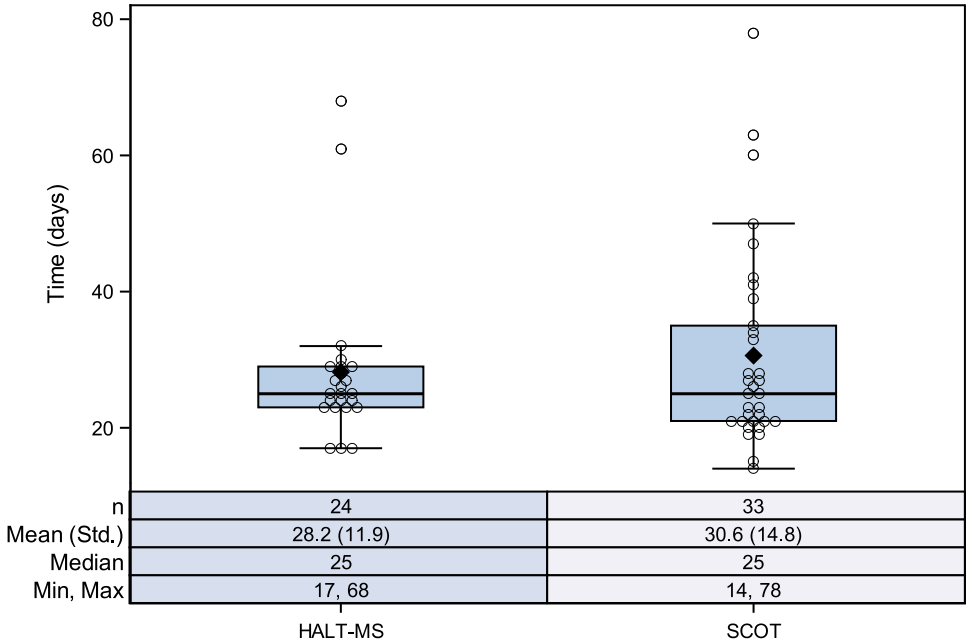

Figure 5. Days from start of collection to transplant by study. The number of days Auto-CD34⁺HPC were stored after apheresis collection, CD34⁺ selection, and cryopreservation, before infusion on the day of transplant, is illustrated for HALT-MS and for SCOT. The number of days was calculated from the start of collection to the day of transplant. Collection may have occurred over a series of days to ensure an adequate dose.

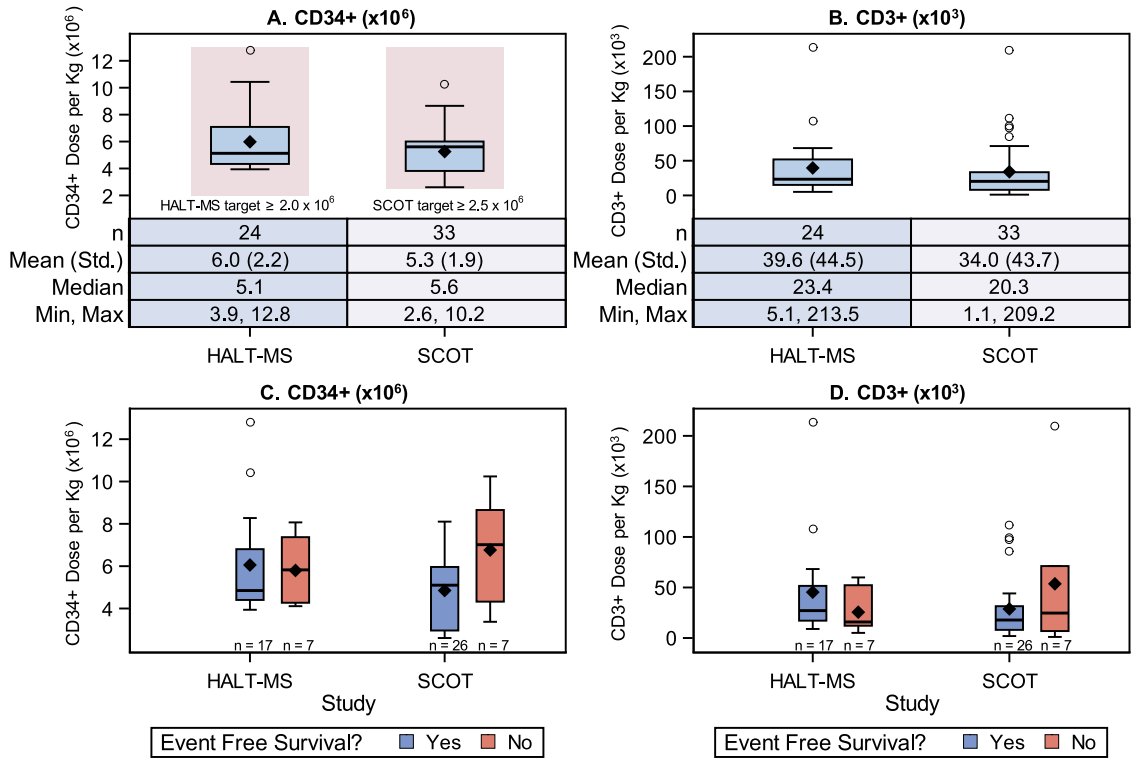

Figure 6. Viable dose per kg administered by study. Graft dose was calculated as viable cells administered/kg RBW. (A,B) Doses meeting or exceeding target CD34+ dose for each respective study are shaded in red (A). Mean doses are denoted with the filled diamond. To achieve these doses, subjects received a mean and median of 2 lots of Auto-CD34⁺HPC per subject (Table 1C). For HALT-MS, endpoint status was event-free survival (EFS), defined as survival without death or disease activity from any one of disability progression, relapse, or new lesions on magnetic resonance imaging (C,D). Participants were evaluated through 5 years post-transplant. For SCOT, endpoint status was EFS, defined as survival without death or organ failure (C,D). Organ failure included respiratory failure defined as decrease in diffusing capacity of the lung for carbon monoxide > 30% or forced vital capacity > 20% from baseline, renal failure defined as requiring dialysis or renal treatment, or cardiac failure defined as left ventricular ejection fraction < 30% or New York Class III. Participants were evaluated through 54 months post-transplant. All HALT-MS subjects had their products processed on the Isolex, whereas SCOT subjects had their products processed on either the CliniMACs or the Isolex device. Of the 8 subjects on SCOT who met endpoint, 1 (12.5%) had their graft prepared on CliniMACs and 7 (87.5%) had their graft prepared on Isolex. Similarly, of the 25 SCOT subjects who did not meet endpoint, 5 (20.0%) had their graft prepared on CliniMACs and 20 (80.0%) had their graft prepared on Isolex.

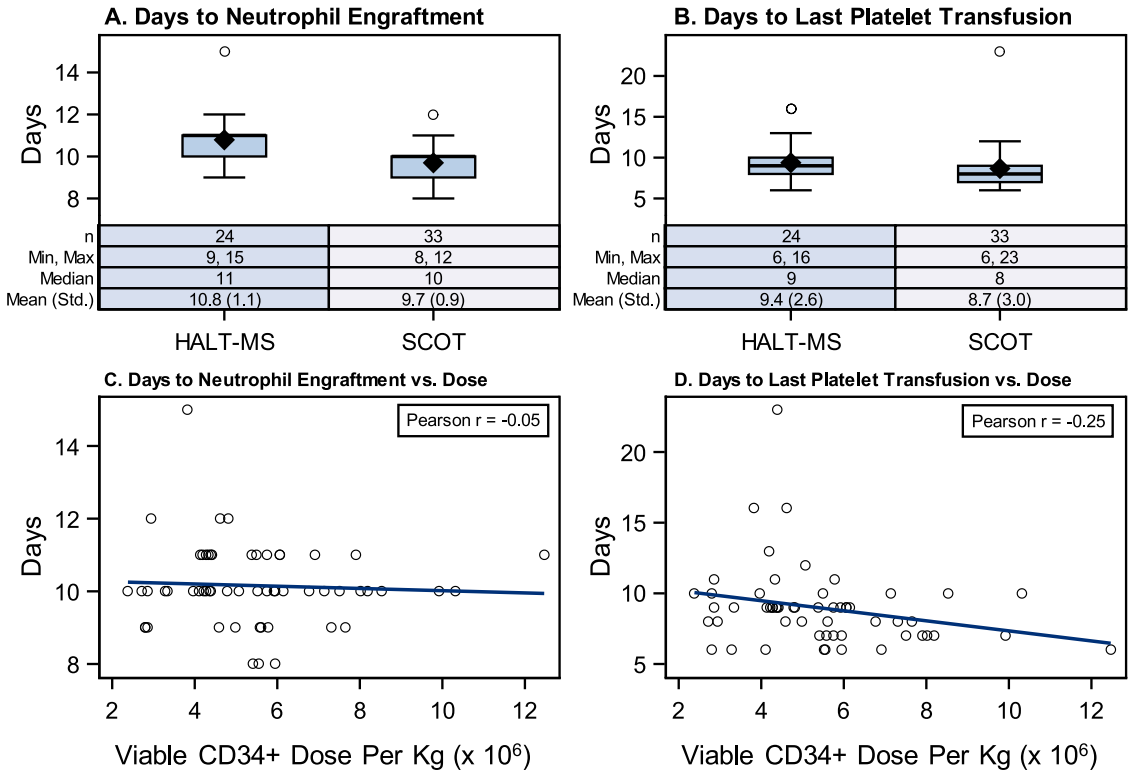

Figure 7. Engraftment kinetics by study. Neutrophil recovery: For HALT-MS, neutrophil engraftment was defined as absolute neutrophil count > 500/μL for 2 consecutive readings. For SCOT, neutrophil engraftment was defined as absolute neutrophil count >500/μL for 3 readings on consecutive days. Platelet recovery: Days to last platelet transfusion was calculated from date of transplant. We note recovery of platelet counts > 20,000/ μL could be expected to be about 7 days after the last platelet transfusion for HALT-MS or SCOT subjects. However, these data were not documented precisely for our studies, as some individuals were discharged from the transplant center and therefore did not receive daily platelet counts in this timeframe. (A and B) The mean is denoted with the filled diamond. (C and D) Pearson correlations are presented as a measure of association.

doses demonstrated a tendency to receive their last platelet transfusion about 2 to 4 days earlier (Figure 7D).

DISCUSSION

For these NIAID studies of autologous HCT for autoimmune diseases, the FDA designated the cell therapy as nonhomologous use and consequently subject to IND regulation. Therefore, we were obligated to ensure harmonized, controlled manufacturing processes at the multiple clinical sites, with production of a uniform cellular therapy product. Product characterization, including development of a potency assay as a marker of biologic activity, begins early in product development and is finally tested during a pivotal clinical trial. Biologic activity is defined by the FDA as ". . .specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data. . .to effect a given result" (21CFR610.1/21CFR600.3(s)). The product is "manufactured" on a consistent basis from lot-to-lot, for all tests in process and lot release. The capacity to demonstrate potency is a prerequisite for product licensure. However, prior clinical studies of autologous HCT for autoimmune diseases were conducted to license the Isolex device under an Investigational Device Exemption and not the $CD34^+$ selected product; consequently, bioassay results in support of a potency assay were not acquired in parallel. A potency assay should correlate with the biologic function of the product and must be reproducible and robust and feasible for use for product release. We considered clonogenic assays for lineage committed hematopoietic progenitors, widely used as a surrogate marker for HPC in the research laboratory. Although such assays more closely fit the FDA definition, they were not well standardized, demonstrated considerable interlaboratory variation, and the turn-around time for final results was not practical. Therefore, as described here, we used viable $CD34^+$ cells as our potency assay.

As evidenced by the timely engraftment kinetics for all subjects on both NIAID studies, successful production, cryopreservation and thawing, and infusion of Auto-$CD34^+$HPC was accomplished at all participating sites. Only a few lots failed to meet the release specifications. These failures primarily consisted of the lots for 1 patient having <70% viable $CD34^+$ cells due to repeated aggregation of cells during processing and 1 incidence of contamination by gram-positive bacteria, found to be skin flora likely acquired during collection.

Since initiation of these NIAID studies in 2005, standards for accreditation and quality management practices for cell processing facilities and transplant centers have continued to evolve [12-14]. Close monitoring is needed for optimization of safety and quality control, and prompt and reproducible engraftment is the best guide to graft potency [15]. A recent study of long-term storage of autologous grafts for >60 months or 12 through ≥60 months demonstrated no negative effect on hematopoietic recovery as compared with storage for ≤12 months [16]. Newer manufacturing systems are semiautomated and closed to a greater extent than the Isolex and CliniMACS devices we used; this should reduce the burden of regulatory oversight needed to ensure comparable cell processing for future multisite clinical trials.

Subjects enrolled on the HALT-MS and SCOT studies had a lower median content of $CD34^+$ cells in their apheresis collections when compared with healthy donors of allogeneic grafts for subjects enrolled on BMT CTN 0303 (median 6.0×10^8 $CD34^+$ cells, with only 1.2% of the collections $< 1 \times 10^8$ $CD34^+$ cells) [17]. Because we did not control apheresis collections for the NIAID studies, in addition to the obvious differences between the healthy donors and patients with autoimmune diseases, this difference may have been because of a variety of study- and center-specific factors, as well as apheresis collection parameters specific to BMT CTN 0303.

For the HALT-MS and SCOT studies, Auto-$CD34^+$HPC doses above 2×10^6/kg were supportive of robust engraftment of neutrophils, independent of cell dose; however, recovery of platelets was slightly enhanced at higher doses. These findings are consistent with previous studies of autologous HCT for a variety of indications [18].

Although the rationale for $CD34^+$ cell selection in the preparation of autologous grafts for HCT for autoimmune diseases is reasonable, namely, to remove immune-competent lymphocytes and other cell types that may potentially be disease-causing, existing data are insufficient to support the necessity of this procedure. One prospective randomized study to investigate the value of $CD34^+$ cell selection [19] and a recent retrospective analysis [20] were inconclusive. Among studies of autologous HCT for MS contemporary to HALT-MS, Atkins et al. (24 subjects) [21] used $CD34^+$ selected grafts. However, others used unmanipulated grafts, including Mancardi et al. (9 subjects) [22], Mancardi et al. (74 subjects) [23], Burman et al. (41 subjects) [24], and Burt et al. (145 subjects) [25]. Clinical outcomes for these studies were promising, comparable with those of HALT-MS. For studies of autologous HCT for systemic sclerosis contemporary to SCOT, van Laar et al. (79 subjects) [26] used $CD34^+$ selected grafts, whereas Burt et al. (17 subjects) [27] used unmanipulated grafts. These studies all reported better long-term survival and/or less progression of impairment for subjects receiving autologous HCT as compared with pulse i.v. cyclophosphamide. In addition to $CD34^+$ cell mobilization and graft preparation, other variables for these studies in MS and scleroderma included transplant regimen and subject disease-specific aspects.

As we have described here, mobilization of autologous PBSCs for both NIAID studies was done successfully using G-CSF and prednisone [1-3], in contrast to use of cyclophosphamide for this purpose in most other published experience for autoimmune disease [21-27]. For patients with scleroderma in particular, due to cardiovascular and other comorbidities, it may be an advantage to limit exposure to cyclophosphamide during mobilization, particularly if the preparative regimen also contains substantial doses of this agent.

Unfortunately, because of the large number of subjects that would be needed for statistical analysis in a randomized clinical trial, investigation of factors potentially important for minor differences in outcomes in autologous HCT for autoimmune diseases, such as $CD34^+$ cell selection or the mobilization regimen, is unlikely to be practicable. However, with a sufficiently large and contemporary database, for example as a collaborative effort of the Center for International Blood and Marrow Transplant Research and European Group for Blood and Marrow Transplantation [28], risk stratification analysis may become feasible. In addition, further study of the immunologic and mechanistic basis of autologous HCT treatment for autoimmune diseases [29-31] may provide the foundation for a rational approach to the improvement of transplant regimens.

ACKNOWLEDGMENTS

The authors thank the patients and their families for their participation in these studies; the technologists and nursing staff of the apheresis centers and cell processing laboratories for HPC collections and graft manufacturing; the research nurses, clinical coordinators, and data technicians for their contributions to patient care and conduct of the protocols; and the Data and Safety Monitoring Board of these studies.

Financial disclosure: The Baxter Isolex 300i Magnetic Cell Selection System, disposable sets, and reagent kits used for the HALT-MS and SCOT studies were supplied by Baxter Healthcare Corporation free of charge. Miltenyi CliniMACS instruments, tubing sets, and reagents used for the SCOT study were provided by Miltenyi Biotec free of charge. This work was sponsored by the Division of Allergy, Immunology, and Transplantation (DAIT), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health. The HALT-MS protocol was conducted by the Immune Tolerance Network (ITN; UM1 AI 109565) and DAIT, NIAID-funded statistical and clinical coordinating centers (UM2 AI 117870). The SCOT protocol was supported by awards to Duke University (N01-AI05419 and HHSN 272201100025C) and DAIT, NIAID-funded statistical and clinical coordinating centers (N01-AI25481, HHSN272200900057C, and 1UMZAI117870). The National Institute of Diabetes and Digestive and Kidney Diseases (DK106829) provided funding support for the collections of the healthy donor G-CSF mobilized apheresis products. The funding organization and sponsor, DAIT, NIAID, National Institutes of Health, participated in design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, and approval of the manuscript; and the decision to submit the manuscript for publication. The opinions expressed are those of the authors and do not represent the position of the NIAID, the National Institutes of Health, or the US Government.

Conflict of interest statement: There are no conflicts of interest to report.

Authorship statement: C.A.K.-T. and S.H. contributed equally to this work.

SUPPLEMENTARY MATERIAL

Supplementary data to this article can be found online at doi:10.1016/j.bbmt.2017.05.018.

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