Cytotherapy, 2014; 16: 965–975

International Society for Cellular Therapy
ISCT

Long-term effects of cryopreservation on clinically prepared hematopoietic progenitor cell products

JACOB M. WINTER[1], PAM JACOBSON[1], BRANDON BULLOUGH[1], AUSTIN P. CHRISTENSEN[1], MICHAEL BOYER[1,2] & JO-ANNA REEMS[1,2]

[1]*University of Utah Cell Therapy and Regenerative Medicine Facility, Salt Lake City, Utah, USA, and* [2]*University of Utah Division of Hematology & Hematologic Malignancies, Salt Lake City, Utah, USA*

Abstract

Background aims. The question of how long hematopoietic progenitor cells (HPCs) destined for clinical applications withstand long-term cryopreservation remains unanswered. To increase our basic understanding about the stability of HPC products over time, this study focused on characterizing long-term effects of cryopreservation on clinically prepared HPC products. *Methods.* Cryovials (n = 233) frozen for an average of 6.3 ± 14.2 years (range, 0.003–14.6 years) from HPC products (n = 170) representing 75 individual patients were thawed and evaluated for total nucleated cells (TNCs), cell viability, viable CD34+ (vCD34+) cells and colony-forming cells (CFCs). TNCs were determined by use of an automated cell counter, and cell viability was measured with the use of trypan blue exclusion. Viable CD34 analysis was performed by means of flow cytometry and function by a CFC assay. *Results.* Significant losses in TNCs, cell viability, vCD34+ cells and CFC occurred on cryopreservation. However, once frozen, viable TNCs, vCD34+ cells and CFC recoveries did not significantly change over time. The only parameter demonstrating a change over time was cell viability, which decreased as the length of time that an HPC product was stored frozen increased. A significant negative correlation (correlation coefficient = −0.165) was determined between pre-freeze percent granulocyte content and post-thaw percent viability (n = 170; $P = 0.032$). However, a significant positive correlation was observed between percent viability at thaw and pre-freeze lymphocyte concentration. *Conclusions.* Once frozen, HPC products were stable for up to 14.6 years at <−150°C. Post-thaw viability was found to correlate negatively with pre-freeze granulocyte content and positively with pre-freeze lymphocyte content.

Key Words: *blood preservation, cell survival, cryopreservation, hematopoietic stem cells, hematopoietic stem cell transplantation/methods*

Introduction

Transplantation of hematopoietic progenitor cells (HPC) is an effective tool in cancer therapy (1–5). HPCs can be collected from peripheral blood by apheresis [HPC(A)], from bone marrow [HPC(M)] and from umbilical cord blood [HPC(CB)] (3). Transplantation typically involves infusing the collected HPC product either fresh or after a short-term (typically less than 6 months) cryopreservation (6,7). As modern clinical techniques extend cancer prognoses and remissions, there is increased potential for storing HPC products for extended times between the time of stem cell collection and possible relapse. Long-term cryopreservation offers a source of HPCs to patients relapsing years after collection (6–9). Long-term cryopreservation is also relevant with respect to cord blood banking, in which HPC(CB) samples may be stored for extended periods of time (10). Numerous studies have indicated that the cryopreservation process itself has detrimental effects on the viability of HPC products (11–13). However, the long-term stability and post-thaw hematopoietic activity of cryopreserved HPC products remain unclear.

Several techniques are available for analyzing HPC products, such as the trypan blue exclusion assay, assessment of viable CD34+ (vCD34+) cells in the sample by means of flow cytometry, and the colony-forming cell (CFC) assay (12). Whereas trypan blue exclusion is the most widely used technique to assess viability, different studies have reported the detrimental effects of cryopreservation in different magnitudes on the basis of which techniques they feature (12,14,15). For instance, vCD34+ and CFC

Correspondence: **Jo-Anna Reems**, PhD, University of Utah, 676 Arapeen Drive, Suite 300, Salt Lake City, UT 84108, USA. E-mail: joanna.reems@hsc.utah.edu

(Received 30 September 2013; accepted 16 February 2014)

http://dx.doi.org/10.1016/j.jcyt.2014.02.005

content appear to be less affected by cryopreservation than is viability (12,14,15).

Galmés *et al.* (16) concluded in their 1999 study that HPC products could not be stored for more than 6 months. However, numerous studies (6–8) have since shown that HPCs can withstand longer freeze durations. In 2011, Cameron *et al.* (6) determined that HPC(A) products can be stored for "at least 5 years with no apparent loss in their ability to support hematopoietic reconstitution in patients after high-dose chemotherapy." This valuable study measures the effects of long-term cryopreservation with the clinical parameter of time to engraftment, but it has a limited data set with respect to freeze duration (mean storage duration of 45 months in the long-term group). A 2002 study by Spurr *et al.* (7) showed that "Hematopoietic stem cell collections can remain adequate for safe transplantation after up to 14 years of cryostorage." However, Spurr *et al.* did not provide extensive details regarding the distribution of freeze duration data points in their long-term test group. In fact, 14 years was the upper limit of their data set, which had a median freeze duration of 9.5 years. Scant research exists that addresses the question of how HPCs withstand freeze durations of longer than 10 years.

The present study builds off of a report by Donnenburg *et al.* (9), who reported that "Bone marrow products can be cryopreserved for more than a decade without apparent loss of progenitor activity." Our study aims to provide a larger sample size and longer duration of cryopreservation than previously reported. Unlike Donnenburg *et al.*, who investigated HPC products frozen in liquid-phase nitrogen, our study involves the long-term storage of HPCs in the vapor phase of a liquid nitrogen freezer, an industry standard. In light of reports demonstrating that the numbers of CFC and CD34+ cells present in a graft are effective predictors of engraftment kinetics and hematopoietic recovery (15,17–20), we used both of these parameters as well as cell viability and viable total nucleated cell count (vTNC) recoveries in our assessment.

Methods

HPC collection

Informed consent from donors for collection of HPCs was obtained according to our institutional review board. HPCs were collected from peripheral blood with the use of the Baxter Fenwall CS3000 PLUS (Baxter Healthcare, Deerfield, IL, USA) or COBE Spectra (Terum BCT, Lakewood, CO, USA) apheresis systems. These systems use centrifugation to separate the mononuclear cell fraction from other peripheral blood components. Bone marrow HPCs were harvested directly from the iliac crest in a surgical setting. HPCs were collected into blood bags containing acid-citrate-dextrose/adenine (Baxter Healthcare) and transported from the clinic to the cell therapy and regenerative medicine facility for processing.

Processing and cryopreservation of HPCs

On arrival of HPCs at the cell therapy and regenerative medicine facility, the cell concentration of the product was assessed with the use of a Sysmex XE5000 blood analyzer (Sysmex America, Inc, Lincolnshire, IL, USA) and cell viability by means of trypan blue (Sigma Aldrich, St Louis, MO, USA) exclusion. The cellular concentration was adjusted to $\leq 300 \times 10^6$ TNC/mL by means of a plasma reduction or volume expansion with Plasmalyte A (Baxter Healthcare). A plasma reduction was accomplished by centrifuging the product at 1400*g* for 12 minutes and removing supernatant with a plasma expressor (Baxter Healthcare) to achieve the desired cell concentration.

After the cell concentration of an HPC product was adjusted, it was cryopreserved in the smallest volume possible at desired cell concentration with a cryoprotectant solution at a final concentration of 10% dimethyl sulfoxide (Protide Pharmaceuticals, Lake Zurich, IL, USA), 10% Plasmalyte A (Baxter Healthcare), with a minimum of 10% autologous plasma or 2.5% human serum albumin (Baxter Healthcare). The products were cryopreserved through the use of controlled-rate freezing (Thermo Electron Corp, Waltham, MA, USA) at a rate of 1°C/min to −10°C, 25°C/min to −60°C, 12°C/min to −18°C, 1°C/min to −40°C and 10°C/min to −85°C. Extension cryovials, 0.5-mL aliquots of representative product, were frozen simultaneously to allow for post-thaw analysis of the transplant product before infusion. The final product and cryovials were stored in vapor-phase liquid nitrogen at <−150°C.

Post-thaw cell counts and trypan blue viability testing

Cryovials (Corning Incorporated, Tewksbury, MA, USA) containing cryopreserved apheresis (n = 143) and marrow (n = 28) products were thawed in a 37°C water bath, and TNC counts were performed with the use of a Sysmex XE-5000 Automated Hematology Analyzer. Because the Sysmex XE5000 does not distinguish viable from non-viable cells, the total number of dead cells (dTNCs) was determined microscopically with the use of trypan blue. Trypan blue staining was performed by diluting cells with