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Cryopreserved mobilized autologous blood progenitors stored for more than 2 years successfully support blood count recovery after high-dose chemotherapy

GIOVANNA CAMERON[1,9], ADISAK TANTIWORAWIT[1], MIKE HALPENNY[2,9], BRENDA LETCHER[3,9], SUE BERRIGAN[4,9], KAREN HINDMARSH[5,9], ANGELINE GIFTAKIS[6,9], JOHANNE FORTIER[7,9], PAMELA O'HOSKI[8,9] & DONNA HOGGE[1]

[1]*The Clinical Cell Therapy Laboratory of the BC Cancer Agency, Vancouver, Canada,* [2]*Stem Cell Processing Laboratory, CBS, Ottawa, Canada,* [3]*Canadian Blood Services, Edmonton, Canada,* [4]*Calgary Laboratory Services, Foothills Medical Centre, Calgary, Canada,* [5]*Saskatchewan Cancer Agency, Saskatoon, Canada,* [6]*Cellular Therapy Laboratory, Cancer Care Manitoba, Winnipeg, Canada,* [7]*Bone Marrow Transplant Laboratory, Centre Hospitalier Affilié Universitaire de Québec, Quebec City, Canada,* [8]*Hamilton Health Sciences, Hamilton, Canada, and the* [9]*Laboratory Practices Committee of the Canadian Blood and Marrow Transplant Group*

Abstract

Background aims. The ability of hematopoietic progenitor cells–apheresis (HPC-A) that have been stored for many years after cryopreservation to reconstitute hematopoiesis following high-dose chemo/radiotherapy has not been well-documented. *Methods.* In this retrospective study, eight Canadian centers contributed data from 53 autologous stem cell transplants (ASCT) performed using HPC-A that had undergone long-term storage (>2 years, range 2–7 years) and 120 ASCT using HPC-A stored for <6 months (short-term storage). *Results.* The doses of nucleated and $CD34^+$ cells per kilogram recipient weight were similar between the short- (mean ± SD, $4.7 \pm 4.9 \times 10^8$ and $6.8 \pm 4.3 \times 10^6$, respectively) and long- ($4.0 \pm 4.9 \times 10^8$ and $6.1 \pm 3.4 \times 10^6$, respectively) term storage groups. The median days to neutrophils (absolute neutrophil count; ANC) $>0.5 \times 10^9$/L (median 11 days for both short- and long-term storage) and platelets $>20 \times 10^9$/L (median 12 and 11 for short- and long-term storage, respectively) post-ASCT were not significantly different between the two groups. When ASCT performed with $<5 \times 10^6$/kg $CD34^+$ cells was compared there was also no difference in ANC or platelet recovery (median 12 days for both after short-term storage, and 12 and 11 days, respectively, after long-term storage). Fourteen HPC-A products stored for >5 years also showed similar count recoveries as the entire long-term storage group (median 11 days for both ANC and platelets). *Conclusions.* Cryopreserved HPC-A can be stored for at least 5 years with no apparent loss in their ability to support hematopoietic reconstitution after high-dose chemotherapy.

Key Words: *cryopreserved, engraftment, long-term storage, short-term storage*

Introduction

Autologous peripheral blood progenitor cells (BPC) mobilized by growth factors alone or in combination with chemotherapy and harvested by apheresis (hematopoietic progenitor cells-apheresis; HPC-A) have been used to support high-dose therapy regimens for patients with advanced cancer for two decades (1–4). The harvested cells are typically mixed with a cryopreservative, frozen and stored at low temperature until required for autologous stem cell transplantation (ASCT) (5,6). Many publications have documented the efficacy of cryopreserved HPC-A in reconstituting hematopoiesis in patients who have received high-dose chemo/radiotherapy (3,4,7–9). The majority of patients will achieve an absolute neutrophil count (ANC) $>0.5 \times 10^9$/L and become platelet-transfusion independent within 14 days of stem cell reinfusion if a dose of $CD34^+$ cells $>2 \times 10^6$/kg or total colony-forming cell (CFC) dose $>5 \times 10^5$/kg is administered (7,8,10–12). On the other hand, variables such as the use of 5% or 10% dimethyl sulfoxide (DMSO) as cryoprotectant and computerization for controlled-rate freezing and overnight storage at 4°C prior to cryopreservation seem to have little impact on engraftment kinetics after short-term storage (5,13,14). When BPC are

Correspondence: Dr **Donna E. Hogge**, Clinical Cell Therapy Laboratory, BC Cancer Research Centre, 675 W 10th Ave, Vancouver, British Columbia, Canada V5Z 1L3. E-mail: dhogge@bccancer.bc.ca

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stored for less than 5 months, a wide range of freezer storage temperatures also appear to be tolerated without loss of graft quality (5,14).

Under most clinical circumstances, patients are referred for BPC mobilization and collection when a clear indication for high-dose chemotherapy exists and a plan is in place to proceed to ASCT shortly after sufficient BPC are collected and cryopreserved. However, circumstances may change and the ASCT may be postponed or cancelled. In addition, there has been recent interest in collecting sufficient BPC to enable a second ASCT to be performed after recovery from the first transplant (15). In some cases BPC are collected and stored for patients in anticipation that the indication for ASCT may exist in the future if the disease status should change. In these latter circumstances HPC-A may remain frozen in storage for long periods of time.

There are little data published on the use of HPC-A for ASCT after storage for more than 2 years. Spurr *et al.* (16) described good colony-forming unit–granulocyte-macrophage (CFU-GM) and viable $CD34^+$ cell recoveries among 40 BPC products stored for 5–14 years, while Donnenberg *et al.* (17) reported similar results for cryopreserved bone marrow. However, these products were not transplanted. Two studies have described the results of transplanting bone marrow cells cryopreserved and stored for more than 2 years (18,19). Although the median time to neutrophil recovery was 19 days and 23 days, respectively, the range was large (10–115 days and 10–119 days) and many of the marrows had been purged using a variety of techniques. Thus these data are not particularly helpful in assessing the likelihood of prompt count recovery after long-term storage of mobilized blood collections that have undergone minimal manipulation. More recently, Liseth *et al.* (20) studied collections from 16 cancer patients that had been stored for 5 years and demonstrated a modest (13.9%) but statistically significant reduction in viable $CD34^+$ cells compared with the same cells stored for 6 weeks. In the same report, 17 myeloma patients received autologous HPC that had been stored for 7–86 months and all showed rapid neutrophil and platelet recovery, suggesting that cells stored for as long as 7 years might successfully support high-dose chemotherapy. However, the sample size in this study was small and the duration of storage highly variable. In addition, no details were given about the conditioning regimen used for ASCT.

The relative lack of published information regarding the clinical use of HPC-A after long-term storage prompted the current study. Data were obtained on ASCT performed using cryopreserved HPC-A from across Canada with the aid of the Laboratory Practices Committee of the Canadian Blood and Marrow Transplant Group (CBMTG). The results of transplants using HPC-A that had been stored for longer than 2 years (long-term storage) were compared with those obtained at the same centers using products stored for less than 6 months (short-term storage).

Methods

This was a retrospective study initiated with the goal of comparing neutrophil and platelet count recoveries for 53 patients who, as part of an ASCT procedure, received cryopreserved HPC-A product(s) that had been stored for greater than 2 years (long-term storage) with 120 transplants performed using cells that had been stored for less than 6 months (short-term storage). Approval to perform this study was obtained from the Clinical Research Ethics Board of the University of British Columbia (Vancouver, Canada) and the institutional review boards from all participating centers. In addition to data from each of the long-term storage ASCT identified in their records, participating centers contacted through the CBMTG Laboratory Practices Committee were asked to contribute data on two short-term storage ASCT that were performed for the same disease indication and for which the $CD34^+$ cell dose was similar to each of the long-term storage ASCT. Centers also provided demographic information on the patients and were asked to specify the BPC mobilization regimen, the high-dose chemotherapy used as conditioning for each ASCT, and post-transplant use of granulocyte–colony-stimulating factor (G-CSF) for each transplanted patient. Time from day of transplant to ANC $>0.5 \times 10^9$/L was documented for all patients. The number of days post-transplant to an unsupported platelet count $>20 \times 10^9$/L was available for 111 patients in the short-term and 51 patients in the long-term storage groups.

Total nucleated cell (TNC) counts were determined for the majority of, and $CD34^+$ cell concentrations on all, the HPC-A prior to cryopreservation. TNC counts were performed using an automated cell counter (Sysmex XE-2100; Sysmex America Inc., Mundelein Illinois, Vancouver, Canada site). $CD34^+$ cell enumeration was performed using flow cytometry and ISHAGE criteria (21,22). Aside from volume reduction and addition of cryoprotectant/additives, no manipulation of the BPC products was performed prior to cryopreservation. The CFC content of HPC-A was determined for 89 of the 173 collections either prior to cryopreservation or at the time of thawing for ASCT. Cells were plated in semi-solid media (product 4434, StemCell Technologies, Vancouver, Canada, or an 'in house' methylcellulose-based preparation at the Quebec center). Total CFC was scored and reported as the number detected per