Low CD34+ Cell Doses are Associated with Increased Cost and Worse Outcome Following of Tandem Autologous Stem Cell Transplantation in Patients with Relapsed or Refractory Germ Cell Tumors

Mustafa A. Hyder,1,3 W. Scott Goebel,2,3 Kirsten D. Ervin,3 Jennifer E. Schwartz,1,3 Michael J. Robertson,1,3 Teresa C. Thakrar,3,4 Costantine Albany,1 and Sherif S. Farag1,3

1Department of Medicine, Division of Hematology and Oncology, 2Department of Pediatrics, Division of Hematology, Indiana University School of Medicine, and 3Bone Marrow and Blood Stem Cell Transplantation Program, and 4Department of Pharmacy, Indiana University Health, Indianapolis, IN

Keywords: Germ cell tumors; stem cell transplantation; mobilization; CD34+ cells; resource utilization

Address for Correspondence:
Sherif S. Farag, MD, PhD, FRACP, FRCPA
Professor of Medicine and Medical Molecular Genetics
Indiana University School of Medicine
980 W. Walnut Street, R3 C414
Indianapolis, IN 46202
United States of America
Email: ssfarag@iu.edu

This is the author's manuscript of the article published in final edited form as:
Highlights
- 23% of GCT patients collect a suboptimal number of PBSC for tandem ASCT using filgrastim
- Low CD34+ cell doses are associated increased transplant costs and worse survival
- Despite higher costs and worse outcome, low CD34 cell doses should not preclude ASCT

ABSTRACT

Tandem autologous stem cell transplantation (ASCT) improves long-term survival of platinum-refractory germ cell tumors (GCT) patients. Studies, predominantly in lymphoma, showed that CD34+ cell doses > 5.0 x 10^6/kg/single transplant lead to decreased resource utilization. As most GCT patients have received prior cisplatin-based treatment, collecting >10x10^6 CD34+ cells/kg is challenging. We analyzed the effect of CD34+ cell dose on resource utilization and outcome of in 131 GCT patients, median age 29.5 (range, 16-58) years, undergoing tandem ASCT. Of 262 individual transplants performed, 120 were performed as inpatient and 142 as planned outpatient. Overall, median CD34+ dose per transplant was 3.1 (0.8 – 16.0) x 10^6/kg, with no significant difference between inpatient and outpatient transplants. Patients were divided into quartiles based on the CD34 cell dose infused: Q1: 0.8-1.9, Q2: 2.0-2.9, Q3: 3.0-4.1, and Q4: 4.2-16.0 x10^6/kg. For all patients, Higher CD34+ cell doses were associated with significantly shorter times to neutrophil (P<0.001) and platelet recovery (P<0.001). For inpatient transplants, higher CD34+ doses were significantly associated with shorter length of hospital stay (LOS) (P<0.001), fewer days of filgrastim (P<0.001), intravenous antibiotic (P=0.012) and antifungal (P=0.03) usage, as well as fewer red blood cell (RBC) (P=0.001) and platelet units transfused (P<0.001), resulting in overall lower cost of care (P<0.001). Of the 142 planned outpatient transplants, 100 admissions were required for a median LOS of 7.0 (1 - 18) days. Although there was no significant difference in the rates of hospitalization between patients in different CD34+ cell dose quartiles, a significant trend was observed for shorter hospitalization (P=0.01), and fewer RBC (P=0.002) and platelet (P=0.005) transfusions with higher CD34+ cell dose quartile. Patients receiving CD34+ cell doses in the lowest dose quartile (Q1) had
significantly worse progression-free survival and overall survival compared to patients receiving higher CD34+ cell doses. Overall, resource utilization and including cost of care, is significantly reduced when patients receive higher CD34+ cell doses, indicating greater efforts to improve PBSC collection in this population are needed.

INTRODUCTION

Germ cell tumors (GCT) are among the most successfully treated solid tumors with overall cure rates nearing 80%, and over 90% for good-risk disease (1, 2). For patients refractory to primary therapy or who relapse after cisplatin-based chemotherapy, treatment with sequential, tandem cycles of high-dose chemotherapy followed by autologous peripheral blood stem cell transplantation (ASCT) results in excellent disease-free and overall survival (3-6), with up to 63% of patients achieving a durable remission at 4-years of follow-up (3, 7, 8).

Previous studies of ASCT primarily in patients with breast cancer and non-Hodgkin’s lymphoma (NHL) identified that infusions of ≥ 5x10^6 cells/kg CD34+ cells result in decreased resource utilization, including reduced requirements for blood transfusions, antibiotics and colony stimulating factors (9). Additionally, further studies showed that the times to hematopoietic engraftment for both platelets and red blood cells was inversely correlated with CD34+ cell dose.(10, 11) As a result, it has since become recommended that the minimum cell dose for ASCT be ≥2.0x10^6 CD34+ cells/kg/transplant, with a recommended ideal target between 3.0x10^6 and 5.0x10^6 CD34+ cells/kg per transplant (12).

In the setting of tandem ASCT for relapsed or refractory GCT, collecting ≥10x10^6 CD34+ cells/kg (5x10^6 CD34+ cells/kg for each transplant) can be challenging since many patients are heavily pre-treated with multiple cycles of chemotherapy, given as front-line or salvage therapy, which increases the odds for poor mobilization CD34+ cell yields (13). Furthermore, unlike the case for multiple myeloma and NHL in which the newer mobilizing agent Plerixafor is approved to augment granulocyte-colony stimulating factor (G-CSF) for peripheral blood stem cell (PBSC)
mobilization, the former drug is not approved for mobilization of PBSC in GCT patients despite previously reported efficacy in this population (14). As a result, lower yields of PBSC may be used in GCT patients. The effect of CD34+ cell doses on resource utilization in tandem transplantation for relapsed or refractory GCT patients has not been previously studied. The aim of this study was to evaluate the effect of CD34+ cell dose on resource utilization and costs among GCT patients undergoing tandem transplantation.

PATIENTS AND METHODS

Patient Selection

We analyzed data for GCT patients treated at Indiana University between January 2010 and September 2014 with high-dose chemotherapy and planned tandem ASCT. Patients included were ≥15 years of age, collected at least 0.8 x 10^6 CD34+ cells/kg per transplant (our institution’s minimum for transplanting GCT patients), and met institutional criteria for acceptable renal and liver function (creatinine < 2.0 mg/dl; AST/ALT < 3 times the upper limit of normal). Notably, no patient was excluded based on an inability to collect at least 0.8x10^6 CD34+ cells/kg per transplant. The study and waiver of consent were approved by the Indiana University-Purdue University (IUPUI) Institutional Review Board.

Mobilization

Patients received filgrastim 10 μg/kg/day subcutaneously (SC) for four days with apheresis taking place on day 5. If additional aphereses were needed, patients continued to receive filgrastim daily until collection was complete at the discretion of the treating physician (generally a maximum of 4 days was allowed for apheresis). The total PBSC cell yield was split in half, and equal CD34+ cell doses were used for each of the tandem transplants in all cases.

Conditioning Regimen and Post-transplantation Care

Tandem transplantation was planned as either outpatient or inpatient depending on the patient’s preference, availability of caregiver support, or less commonly, if the patient’s medical
condition indicated that inpatient care would be safer. The preparative regimen for each transplant included etoposide 750 mg/m² and carboplatin 700 mg/m² on days -5, -4 and -3, as previously reported (3). Peripheral blood stem cells were infused on Day 0.

On day 0, following infusion of PBSC, patients received filgrastim 5 μg/kg/day SC until the absolute neutrophil count (ANC) reached ≥ 0.5 x 10⁶/l for at least 2 consecutive days. Intravenous (IV) vancomycin, oral (PO) ciprofloxacin, acyclovir, and fluconazole were used for prophylaxis of infection. Patients who developed neutropenic fever were empirically treated with IV cefepime (or aztreonam in case of allergy), and subsequent antibiotic coverage was modified according to blood culture results as appropriate. IV antifungal agents were substituted for fluconazole for persistent neutropenic fever or suspected fungal infections. Packed red blood cells were transfused for a hemoglobin (Hb) < 7 gm/dl (or at a higher Hb if patient was symptomatic); platelets were transfused for a count < 10 x 10⁹/l. Outpatients were admitted if they developed neutropenic fever or a toxicity that was difficult to adequately manage as an outpatient at the attending physician’s discretion. After patients were discharged from the first transplant, they were readmitted 7 days later for the second transplant.

Time to neutrophil engraftment was calculated from day 0 until the first day of three consecutive days when the ANC was ≥ 0.5 x 10⁶/l, and the time to platelet engraftment was calculated from day 0 until the first of seven consecutive days when the platelet count was ≥ 20 x 10⁹/l without platelet transfusion.

Data Collection & Analysis

Patients were grouped into quartiles on the basis of the infused CD34⁺ cell/kg dose per transplant, Quartile 1 (Q1): 0.8-1.9 x 10⁶/kg; Quartile 2 (Q2): 2.0-3.0 x 10⁶/kg; Quartile 3 (Q3): 3.1-4.1 x 10⁶/kg; Quartile (Q4): 4.2-16.0 x 10⁶/kg; and analyzed separately for planned inpatient (IP) and planned outpatient (OP) transplants. For IP transplants, data collected included days till neutrophil and platelet engraftment, length of hospital stay (LOS), post-transplant doses of filgrastim, IV antibiotics, IV antifungals, and number of blood products transfused. For OP, data
collected included days till neutrophil and platelet engraftment, hospital admissions needed and associated LOS, and the number of blood products transfused.

**IP Cost of Care**

Because of the difficulty in obtaining accurate cost data for OP transplants, the cost of care was calculated only for IP transplants. For each IP transplant, a cost of care value was calculated for post-transplant care, including doses of filgrastim, IV antibiotics (including IV antivirals), IV antifungals, units of red cell and platelet products, total parenteral nutrition (TPN), and days of hospitalization. Costs for pharmaceuticals, including filgrastim, IV antibiotics, IV antifungals and TPN were based on wholesale generic drug prices obtained from Lexicomp Online (http://www.wolterskluwercdi.com/lexicomp-online/), and were calculated based on standard daily dosage for a 70 kg individual. Blood product costs were estimated from blood-bank costs.

Cost of hospitalization was based on the mean daily variable direct cost (VDC) for 50 randomly selected inpatients included in the study. The VDC was obtained from internal billing records and included costs of direct nursing care, pharmacy services, laboratory studies, blood bank services, any operating room costs, radiology, cardiology services, respiratory therapy, rehabilitation services, and supplies. All pharmacy and blood product costs were then subtracted from the VDC to determine the daily cost of hospitalization. The unit cost of the individual items used in the calculation of total cost is shown in Table 1.

**Statistical Analysis**

Baseline variables by CD34+ cell dose quartiles were compared using the Kruskal-Wallis test. Estimates of times to neutrophil and platelet engraftment were calculated using the Kaplan-Meier method and comparisons by CD34+ cell dose quartiles used the Log-rank test. Resource usage was compared by CD34+ cell dose quartiles using the Kruskal-Wallis test. To identify significant independent variables that may be associated with total costs (combined cost of the two transplants) for IP tandem transplants, multivariable analysis was performed. Factors
included in the analysis were age at first transplant, year of transplant, Karnofsky performance status (KPS), International Germ Cell Consensus Classification (IGCCC) risk category (1), lines of chemotherapy prior to transplantation, number of cycles of platinum-based chemotherapy, and CD34+ cell dose quartile. Multivariable linear regression analysis was performed using a backward stepwise method. The Kaplan-Meier method was used to calculate progression-free survival (PFS) and overall survival (OS) with time to events being from the first of the tandem transplants, and the Cox proportional hazards regression was used to determine hazard ratios. All $P$-values were 2-sided and considered significant if $P<0.05$. Data were analyzed using SPSS version 23.0 (Armonk, NY). Cumulative incidence of relapse (CIR) and non-relapse mortality (NRM) was treated as competing events and calculated using R version 3.3.1 (The R Foundation).
RESULTS

Patient Characteristics

One hundred and fifty-one relapsed or refractory GCT patients underwent ASCT from January 2010 to September 2014. Of these, 20 patients were excluded from analysis because 15 patients underwent only one of the tandem transplants due to excessive toxicity, disease-progression or death before the planned second transplant, and 5 had incomplete data for at least one of the two tandem transplants. Therefore, 131 patients who completed tandem transplantations were included in the analysis for a total of 262 separate ASCT. All patients received both transplants as either IP (n=120) or OP (n=142).

The median age for all included patients was 29.5 years (range, 16 - 58 years), and the overall median CD34+ cell dose per transplant was 3.1 (0.8 – 16.0) x10^6/kg. Table 2 shows the baseline variables of patients according to CD34+ cell dose quartile. As shown, while patients in the lowest CD34+ cell dose quartile were somewhat older, there were no significant differences across the different dose quartiles with respect to gender, KPS, IGCCC risk stage, number of lines of prior therapy, and number of cycles of platinum based chemotherapy. On multivariable logistic regression analysis that included KPS, lines of chemotherapy before transplant, and number of platinum-based cycles (≤4 cycles vs. >4 cycles), no factor was significantly associated with CD34+ cell dose above or below the median (results not shown). As may be expected, there was a significant difference in the number of apheresis days across the CD34+ cell dose quartiles \( (P<0.001) \), with more days required to complete collection in the lower CD34+ cell quartiles. Compared to those undergoing planned OP transplantation, patients receiving IP transplants were significantly older (median age 35 [range, 18-58] vs. 27 [range, 16-57] years; \( P<0.001 \)), required more apheresis days (median 2 [range, 1-6] vs. 1 (range, 1-3); \( P=0.006 \)), and had a lower median CD34+ cell dose per transplant (2.9 [0.8-16] vs. 3.2 [1.0-10.0] x10^6/kg; \( P=0.015 \)).
Neutrophil and Platelet Engraftment

Figures 1 shows the neutrophil and platelet recovery by CD34+ cell dose quartile including all transplants for patients receiving both planned IP and OP transplants. As shown, higher CD34+ cell doses were associated with significantly faster neutrophil (Figure 1A) and platelet recovery (Figure 1B). Additionally, there were no significant differences in the times to neutrophil ($P=0.966$) and platelet ($P=0.817$) recovery when comparing between the first and second transplants (data not shown). Of note, despite the CD34+ cell doses infused in patients in Q1 (median CD34+ cell dose $1.45 \times 10^6$/kg, 25th and 75th percentiles CD34+ cell doses $1.2 \times 10^6$/kg and $1.6 \times 10^6$/kg respectively, with one patient receiving $0.8 \times 10^6$/kg and 5 patients receiving $1.1 \times 10^6$/kg), there were no engraftment failures.

Resource Utilization, Hospitalization days, and Overall Cost for planned IP transplants

Table 3 shows the number of days of filgrastim usage post-transplantation, usage of IV antibiotics and antivirals, IV antifungals, TPN, and the number of units of red blood cells and platelets transfused per transplant for each CD34+ cell dose quartile. As shown, higher CD34+ cell doses were associated with fewer days of filgrastim usage ($P<0.001$), days of IV antibiotics and antivirals ($P=0.012$) and IV antifungals ($P=0.03$), and fewer numbers of RBC ($p=0.001$), and platelet ($p<0.001$) transfusions. No significant difference, however, was found in the number of days of TPN use ($P=0.073$). For planned IP transplants, there was also a significant difference in length of hospital stay, with reduced duration of hospitalization with increasing CD34+ cell doses ($P<0.001$ for trend) (Table 3).

The reduced resource utilization and days of hospitalization with higher doses of CD34+ cells translated to lower overall IP costs per transplant. Although, overall, the median cost of the first transplants was significantly higher than that of the second transplant by $2,166$ ($P=0.016$), the cost per planned IP transplant per CD34+ cell dose quartile is shown in Figure 2. As shown, there was an incremental reduction in median overall cost for each higher CD34+ cell quartile ($P<0.001$ for trend). The difference in median cost of a single transplant between the lowest and
highest quartiles was $9,673. Correcting for multiple comparisons, pairwise analysis showed significant differences in transplant cost between the lowest CD34+ cell dose quartile (Q1) and Q3 ($P=0.002$) and Q4 ($P<0.001$), as well as between Q2 and Q4 ($P=0.025$). On multivariable linear regression analysis that included age at transplant, KPS, lines of prior therapy, number of cycles of prior platinum therapy, and CD34+ cell dose quartile, worse KPS ($β$-coefficient = $-364$; $p=0.003$) and lower CD34+ cell quartile ($β$-coefficient = $-5,584$; $p<0.001$) were the only variables significantly associated with higher costs.

**Resource utilization for planned OP transplants**

For patients undergoing planned OP transplants, accurate overall costs were difficult to obtain. Therefore, analysis for these patients was restricted to hospitalization rates and days, as well as number of units of blood products transfused. Overall, 8 (11%) of 71 patients who were planned to receive tandem outpatient transplantation were able to undergo both transplants completely as outpatient. As shown in Table 4, although there was no significant difference in the rates of hospitalization between CD34+ cell dose quartiles, a significant trend was observed for shorter durations of hospitalization with higher CD34+ cell dose quartile ($P=0.01$). Additionally, as with IP transplants, there were significantly fewer RBC ($P=0.002$) and platelet ($P=0.005$) transfusions with increasing CD34+ cell dose quartile.

**Non-relapse mortality, PFS, OS, and relapse/progression**

While no patient died during first transplant, 2 of the 30 patients in the lowest CD34+ cell dose quartile (Q1) died during the second transplant, one of sepsis on day +5 and another on day +13 of sinusoidal obstruction syndrome despite successful engraftment. No patient died during transplantation in higher CD34+ cell dose quartiles ($P=0.051$, Fisher’s exact test). Figures 3A and 3B show the PFS and OS, respectively, for all patients by CD34+ cell dose quartile. As shown, patients in the lowest CD34+ cell quartile have the shortest PFS and OS, with similar results for patients in higher CD34+ quartiles (Q2-Q4). The median PFS for patients in Q1 was 348 days, with the median not being reached for patients in each of Q2 to Q4. The 4-
year PFS for patients in Q1 was 38% (95% CI: 20% to 56%), Q2 was 61% (95% CI: 43% to 79%), Q3 was 65% (95% CI: 49% to 81%), and Q4 was 64% (95% CI: 48% to 80%) ($P=0.087$). As the PFS was similar for patients in Q2, Q3 and Q4, the PFS for these patients was compared to that of patients in Q1. As shown in Figure 3A, patients receiving CD34+ cell doses <2.0x10$^6$/kg (Q1) had a significantly lower PFS compared with those receiving higher cells doses (Q2-Q4)($P=0.007$).

Similarly, the median OS for patients in Q1 was 577 days, while the median survival was not reached for patients in each of the higher CD34+ cell dose quartiles (Q2 to Q4). The 4-year OS in Q1 was 45% (95% CI: 26% to 63%), in Q2 was 62% (95% CI: 44% to 80%), in Q3 was 70% (95% CI: 54% to 86%), and in Q4 was 60% (95% CI: 40% to 80%) ($P=0.109$). Compared to those receiving higher CD34+ cell doses (Q2-Q4), patients receiving CD34+ cells <2.0x10$^6$/kg (Q1) had significantly lower OS ($P=0.020$) (Figure 3B).

In multivariable Cox regression models, including KPS (greater or less than 70), IGCCC risk stage, platinum refractory disease (defined as tumor progression within 4 weeks after platinum-based chemotherapy) and CD34+ cell dose quartile (Q1 versus Q2-Q4), KPS<70 (HR: 4.17 [CI: 1.81-9.59]; $P=0.001$), platinum refractory disease (HR: 2.13 [CI: 1.11-4.09]; $P=0.022$), and CD34+ dose quartile 1 (HR: 2.42 [CI: 1.21-4.85]; $P=0.013$) were associated with inferior PFS. Similarly, KPS<70 (HR 5.07 [95% CI: 2.14-12.04]; $P<0.001$), CD34+ dose quartile 1 (HR 2.48 [95% CI: 1.21-5.08]; $P=0.013$) and platinum-refractory disease (HR 1.98 [95% CI: 1.01-3.89]; $P=0.046$) were all associated with worse OS.

The risk of relapse was highest in patients receiving the lowest CD34+ cell dose quartile. The 4-year cumulative incidence of relapse (CIR) for patients in Q1 was 51% (95% CI: 31% to 70%), Q2 was 36% (95% CI: 18% to 53%), for Q3 was 27% (95% CI: 12% to 42%), and for Q4 was 33% (95% CI: 17% to 49%). As shown in Figure 3C, compared to that of patients receiving higher CD34+ cell doses, the CIR was higher for patients receiving CD34+ cell doses in the lowest quartile (Q1), although this did not reach statistical significance ($P=0.085$).
DISCUSSION

GCT patients present a situation in which upfront treatments are extremely effective at curing the disease, but also highly myelotoxic. By the time transplantation is warranted, many patients have received multiple cycles of chemotherapy, which compromises the ability to collect the recommended number of CD34+ cells for tandem transplantation. As found in our study, 23% of patients collected below the minimum recommended cell dose of 2.0x10^6 CD34+ cells/kg/transplant, and fewer than 50% collected above the preferred target doses of 3.0-5.0x10^6 CD34+ cells/kg per transplant.

Confirming the results of prior studies done in other diseases, our results show an inverse correlation between resource usage and cell doses infused. In one study by Schulman et al, including patients who were primarily diagnosed with NHL and breast cancer, those receiving above a threshold of 5.0 x 10^6/kg or higher CD34+ cells were more likely to have fewer RBC and platelet transfusions, use fewer antibiotics and antifungals, and have shorter hospital stays than those receiving fewer CD34+ cells (9). Similarly, Limat et al. used a threshold of 5.0x10^6 cells/kg in their cost analysis of NHL patients, and showed a reduction in hospitalization, anti-infective use, and blood product use among those in the higher cell dose category (15). In the same study, there was an overall cost savings of $4,210 (as of the year 2000) owing primarily to a reduction in hospitalization costs in those receiving >5x10^6 CD34+ cells/kg. Schulman et al showed a greater overall decrease in cost of $9,134 (as of the year 1999) in univariable analysis, with fewer hospitalization days also accounting for the major proportion of savings in patients in the higher cell dose category. In a study by Baron et al, an overall savings of EU 4,693 (as of year 2004) in breast cancer patients who received >5x10^6 cells/kg (16).

Our study is the first in GCT patients and also the only one to consider the significance of lower CD34+ cell dose thresholds, which are more realistic in our patient population as only 18% of patients received over 5.0x10^6 CD34+ cells/kg per transplant. In our analysis, there was an incremental decrease in total direct costs with each increasing cell dose quartile, with a
difference between the highest and lowest quartiles of $9,673/transplant. Furthermore, along with KPS, lower CD34+ cell dose was an independent factor impacting higher total cost of tandem transplantation. Although our cost value is similar to those presented in previous studies, it is important to note that it is difficult to compare directly across studies given differences in transplant protocols and physician practice across institutions, usage of billing costs versus charges to patient in calculation, and changes in costs of variables over time and place (17). Further, we acknowledge that as each patient received two transplants, the power of our analyses may have been increased because each of the tandem transplants was individually included.

Notably, we did not include the cost of apheresis into the overall cost of care as our data collection was limited to the post-transplant period, but it is reasonable to infer that a similar cost trend would be observed as patients who ended up collecting fewer cells had also undergone more apheresis procedures. Therefore, the cost differential between CD34+ quartiles may in fact be even higher than what we observed.

Our study also found that CD34+ cell dose in the range of the lowest quartile to be associated with shorter OS and PFS, and a higher CIR compared with higher cell dose quartiles. Overall, few studies have looked at survival as a function of CD34+ cell dose in the ASCT setting, given that transplantation is seen as a means of reconstituting the hematologic system after high-dose chemotherapy rather than functioning as an effector of a graft versus tumor response as in allogeneic transplants. In reported studies in the autologous transplantation setting, Baron et al noted a 1-year treatment related mortality (TRM) of 16% in breast cancer patients receiving $<5 \times 10^6$ CD34+ cells/kg compared with 0% in those receiving higher cell doses as well as an OS at 5 years of 45% compared with 60% (16). Schulman et al noted a 100-day mortality of 5.9% in the lower cell dose group, versus 2.7% in the higher cell dose group (9). An improved outcome in PFS and OS patient with multiple myeloma with higher CD34+ cells mobilized has also been reported,(18) although no such relationship was noted in patients with non-Hodgkin lymphoma (15). Notably, in our study we observed inferior PFS and
OS in patients receiving the lowest CD34 cell doses (<2x10^6 CD34+ cells/kg), which appeared to be associated with a trend towards higher 100-day NRM and risk of relapse. The basis of this association is currently unclear but does not appear to be related to graft failure. In fact, the patients infused with the lowest CD34+ cell dose (0.8x10^6/kg per transplant) successfully engrafted following both tandem transplants. Importantly, it is not clear if the lower numbers of CD34+ cells collected and infused are in some way a surrogate for additional adverse risk factors not measured by other clinical parameters. Indeed, we could not identify any disease or treatment related-risk factors that were associated with poorer CD34+ cell mobilization and cell yield. On the other hand, it is possible that higher CD34+ cell doses infused may have resulted in better immune reconstitution post-transplantation which could have contributed to reduced relapse, as previously reported in the autologous transplant setting in other diseases (19, 20). Unfortunately, we do not have data on immune reconstitution for the patients included in this study to investigate this possibility. Additional studies will be beneficial in elucidating the exact mechanisms by which CD34+ cell dose is linked to survival and relapse, if at all, and if using novel mobilizing agents to increase CD34+ cell doses can improve survival. Notwithstanding the increased costs and inferior survival associated with very low doses of CD34+ cells, it is important to emphasize that engraftment is still possible even with very low doses (e.g., 0.8-1.9 x10^6/kg per transplant) and tandem transplantation should not be withheld from GCT patients who fail to collect well as a significant proportion can be cured despite poor collections.

Overall, our results show that lower CD34+ cell doses are associated with an increase in resource utilization and costs of tandem autologous PBSC transplantation for GCT patients, and may potentially be associated with a higher risk of relapse and worse survival. Prospective studies will be helpful in determining if augmenting the CD34+ cell dose infused, such as with upfront use of newer mobilizing strategies, including plerixafor, which has demonstrated success in GCT patients (14), will result in improvements costs and outcome, and if the increased cost of mobilization associated with these interventions would be justified.
References


Figure 1. (A) Times to neutrophil engraftment, (B) Time to platelet engraftment

Figure 2. Box plots of overall cost for inpatient (IP) tandem transplants by CD34+ cell dose quartile: $P<0.001$ for trend. Pairwise comparisons: Q1 v Q2, $P=0.17$; Q1 v Q3, $P=0.002$; Q1 v Q4, $P<0.001$; Q2 v Q3, $P=1.0$; Q2 v Q4, $P=0.025$; Q3 v Q4, $P=0.47$ (all $P$-values corrected for multiple comparisons).

Figure 3. (A) Progression-free survival, (B) Overall survival, and (C) Cumulative incidence of relapse by CD34+ cell dose
Table 1. Individual item costs of IP care

<table>
<thead>
<tr>
<th>Resource</th>
<th>Unit cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filgrastim (per dose)</td>
<td>$550</td>
</tr>
<tr>
<td>Hospitalization (per day)</td>
<td>$970</td>
</tr>
<tr>
<td>IV antibiotics and antivirals (per day)</td>
<td>$106</td>
</tr>
<tr>
<td>IV antifungals (per day)</td>
<td>$ 47</td>
</tr>
<tr>
<td>Platelet (per unit)</td>
<td>$400</td>
</tr>
<tr>
<td>Red blood cells (per unit)</td>
<td>$500</td>
</tr>
<tr>
<td>Total parenteral nutrition (TPN) (per day)</td>
<td>$ 53</td>
</tr>
</tbody>
</table>
### Table 2. Baseline characteristics of patients undergoing planned tandem transplants

CD34+ cell dose quartile (Q): range (x 10^6/kg per transplant)

<table>
<thead>
<tr>
<th></th>
<th>Q1: 0.8 - 1.9</th>
<th>Q2: 2.0 - 2.9</th>
<th>Q3: 3.0 - 4.1</th>
<th>Q4: 4.2 - 16.0</th>
<th>P-value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>30</td>
<td>32</td>
<td>35</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>Age *</td>
<td>39 (16 - 58)</td>
<td>29 (16 - 55)</td>
<td>31 (17 - 57)</td>
<td>27 (16 - 57)</td>
<td>0.05</td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>97</td>
<td>97</td>
<td>97</td>
<td>100</td>
<td>0.779</td>
</tr>
<tr>
<td>KPS *</td>
<td>90 (70 – 100)</td>
<td>90 (50 – 100)</td>
<td>100 (60 – 100)</td>
<td>90 (70 – 100)</td>
<td>0.11</td>
</tr>
<tr>
<td>IGCCC risk stage, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.936</td>
</tr>
<tr>
<td>Good</td>
<td>9 (30%)</td>
<td>13 (41%)</td>
<td>16 (46%)</td>
<td>13 (38%)</td>
<td></td>
</tr>
<tr>
<td>Intermediate</td>
<td>2 (7%)</td>
<td>2 (6%)</td>
<td>4 (11%)</td>
<td>3 (9%)</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>17 (57%)</td>
<td>16 (50%)</td>
<td>14 (40%)</td>
<td>18 (53%)</td>
<td></td>
</tr>
<tr>
<td>Unavailable</td>
<td>2 (7%)</td>
<td>1 (3%)</td>
<td>1 (3%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Lines of prior chemo *</td>
<td>1 (1 – 3)</td>
<td>2 (1 – 2)</td>
<td>2 (1 – 4)</td>
<td>2 (1 – 3)</td>
<td>0.097</td>
</tr>
<tr>
<td>Platinum Sensitive (% in quartile)</td>
<td>54%</td>
<td>71%</td>
<td>71%</td>
<td>65%</td>
<td>0.463</td>
</tr>
<tr>
<td>Cycles of platinum *</td>
<td>4 (2 – 8)</td>
<td>4 (3 – 8)</td>
<td>4 (3 – 11)</td>
<td>5 (3 – 7)</td>
<td>0.905</td>
</tr>
<tr>
<td>Number of apheresis days, median (range)</td>
<td>2.5 (1 – 6)</td>
<td>2 (1 – 3)</td>
<td>1 (1 – 3)</td>
<td>1 (1 – 3)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*values represent medians (ranges)

**P-values calculated using Kruskal-Wallis test, except for nominal variables (gender, IGCCC risk, platinum sensitivity), which were calculated using Chi-squared method.

### Table 3. Resource usage for IP transplants

CD34+ cell dose quartile (Q): range (x 10^6/kg)

<table>
<thead>
<tr>
<th></th>
<th>Q1: 0.8 - 1.9 (n=35)</th>
<th>Q2: 2.0 - 2.9 (n=28)</th>
<th>Q3: 3.0 - 4.1 (n=33)</th>
<th>Q4: 4.2 - 16.0 (n=24)</th>
<th>P-value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filgrastim, days*</td>
<td>13 (6 - 19)</td>
<td>12 (10 - 13)</td>
<td>12 (6 - 16)</td>
<td>10 (0 - 12)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IV Antibiotics, days*</td>
<td>21 (11 - 45)</td>
<td>17 (11 - 35)</td>
<td>18 (10 - 39)</td>
<td>14.5 (5 - 29)</td>
<td>0.012</td>
</tr>
<tr>
<td>IV Antifungals, days*</td>
<td>5 (0 - 12)</td>
<td>0 (0 - 11)</td>
<td>0 (0 - 14)</td>
<td>0 (0 - 11)</td>
<td>0.03</td>
</tr>
<tr>
<td>RBC Transfusions, n*</td>
<td>4 (0 - 11)</td>
<td>2 (0 - 9)</td>
<td>2 (0 - 6)</td>
<td>1 (0 - 5)</td>
<td>0.001</td>
</tr>
<tr>
<td>Platelet Transfusions, n*</td>
<td>7 (2 - 25)</td>
<td>6 (2 - 39)</td>
<td>4 (1 - 18)</td>
<td>4 (1 - 10)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TPN, days*</td>
<td>0 (0 - 14)</td>
<td>0 (0 - 4)</td>
<td>0 (0 - 7)</td>
<td>0 (0 - 6)</td>
<td>0.073</td>
</tr>
<tr>
<td>Days of hospitalization, n*</td>
<td>19 (10-44)</td>
<td>18 (15-31)</td>
<td>17 (14-27)</td>
<td>12 (12-21)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*values represent medians (ranges)

**P-value calculated using Kruskal-Wallis test
Table 4. Hospitalization rate and resource usage for OP transplants

<table>
<thead>
<tr>
<th>CD34+ cell dose quartile (Q): range (x 10^6/kg)</th>
<th>P-value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1: 0.8 - 1.9</td>
<td></td>
</tr>
<tr>
<td>Q2: 2.0 - 2.9</td>
<td></td>
</tr>
<tr>
<td>Q3: 3.0 - 4.1</td>
<td></td>
</tr>
<tr>
<td>Q4: 4.2 - 16.0</td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td></td>
</tr>
<tr>
<td>Readmissions, %</td>
<td></td>
</tr>
<tr>
<td>LOS, days*</td>
<td></td>
</tr>
<tr>
<td>RBC Transfusions, n*</td>
<td></td>
</tr>
<tr>
<td>Platelet Transfusions, n*</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Q1: 0.8 - 1.9</th>
<th>Q2: 2.0 - 2.9</th>
<th>Q3: 3.0 - 4.1</th>
<th>Q4: 4.2 - 16.0</th>
<th>0.7</th>
<th>0.01</th>
<th>0.002</th>
<th>0.005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>25</td>
<td>36</td>
<td>38</td>
<td>43</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Readmissions, %</td>
<td>68</td>
<td>75</td>
<td>63</td>
<td>72</td>
<td>0.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOS, days*</td>
<td>9 (3 – 18)</td>
<td>9 (3 – 16)</td>
<td>6 (1 – 12)</td>
<td>6 (3 – 13)</td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBC Transfusions, n*</td>
<td>2 (0 – 6)</td>
<td>2 (0 – 6)</td>
<td>0.5 (0 – 3)</td>
<td>1 (0 – 4)</td>
<td>0.002</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet Transfusions, n*</td>
<td>4 (2 – 25)</td>
<td>3 (1 – 8)</td>
<td>3 (1 – 10)</td>
<td>3 (1 – 11)</td>
<td>0.005</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*values represent medians (ranges)

**P-value calculated using Kruskal-Wallis test
Figure 1

(A)

CD34+ cell dose quartile

- 4.2-16.0x10⁶/kg
- 3.0-4.1x10⁶/kg
- 2.0-2.9x10⁶/kg
- 0.8-1.9x10⁶/kg

Cumulative incidence of neutrophil engraftment

Days post-transplantation

P<0.001

(B)

CD34+ cell dose quartile

- 4.2-16.0x10⁶/kg
- 3.0-4.1x10⁶/kg
- 2.0-2.9x10⁶/kg
- 0.8-1.9x10⁶/kg

Cumulative incidence of platelet engraftment

Days post-transplantation

P<0.001
Figure 2

Cost of IP Transplants

CD34+ Cell Dose Quartiles (x 10^6/kg/transplant)
Figure 3A

CD34+ cell dose (Q1) <2x10^6/kg

CD34+ cell dose (Q2-Q4) ≥2x10^6/kg

P = 0.020

66% (95%CI: 56% - 75%)

45% (95%CI: 26% - 63%)

Figure 3B

CD34+ cell dose (Q1) <2x10^6/kg

CD34+ cell dose (Q2-Q4) ≥2x10^6/kg

P = 0.007

64% (95%CI: 54% - 73%)

38% (95%CI: 20% - 56%)
Figure 3C

Cumulative incidence of relapse/progression

- 51% (95% CI: 31% - 70%)
- 32% (95% CI: 22% - 41%)

Days post-transplantation

- CD34+ cell dose (Q2-Q4) ≥2x10⁶/kg
- CD34+ cell dose (Q1) <2x10⁶/kg

P=0.085