Open Access

OBM Transplantation

LIDSEN Publishing Inc.

Original Research

Mobilization of Autologous Peripheral Blood Stem Cells (PBSC) in CD20+ Lymphoma Patients Using RICE, Granulocyte-Colony Stimulating Factor (G-CSF) and Plerixafor

Leona A. Holmberg [1, 2, *], Michael Linenberger [1, 2], Laura Connelly-Smith [1, 2]

1. Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA; E-Mails: lholmber@fredhutch.org; mlinenbe@fredhutch.org; lsconnel@fredhutch.org
2. Department of Medicine, University of Washington, Seattle, WA, USA

* **Correspondence:** Leona A. Holmberg; E-Mail: lholmber@fredhutch.org

Academic Editor: Kamyar Afshar

OBM Transplantation
2023, volume 7, issue 1
doi:10.21926/obm.transplant.2301178

Received: May 06, 2022
Accepted: February 17, 2023
Published: March 30, 2023

Abstract

RICE is salvage therapy for treating CD20+non-Hodgkin lymphoma (NHL). It is combined with G-CSF to collect autologous peripheral blood stem cells (aPBSC). Little data exists, though, on the combination of G-CSF and Plerixafor after RICE in mobilizing adequate number of CD34 cells and the product's immune content. We report on the results of twenty CD20+ NHL patients after RICE, G-CSF and Plerixafor were given to collect aPBSC. The median number of cells collected was 12.92 × 10^6 CD34 cells/kg (range 5.44-83.76). Plerixafor toxicity included diarrhea (n = 5) and injection-site irritation (n = 1). Seventeen patients collected; fifteen patients did so in one session. Two patients with CLL/Richter or transformed follicular had positive-flow products. Addition of Plerixafor to G-CSF increased by 2.6-9 folds the number of blood CD34 cells. Sixteen patients went to ASCT, with a median of 7.29 × 10^6 CD34 cells/kg infused. The median engraftment time post-ASCT for neutrophils was 12 (range 10-19), for platelets ≥20K 11 (range 0-19) and ≥50K 16.5 (range 11-42) days. There were no graft failures. In APBSC product, there was no evidence of NK or LAK lytic activity (n = 10), only LAK activity (n = 4) and both LAK and NK activity (n = 2). Blood NK activity was common on day +28 post-ASCT. There was no significant correlation between apheresis product and the number of

blood immune cells post- ASCT or relapse. Addition of Plerixafor to RICE/G-CSF is well tolerated. The majority of patients collected aPBSC in one session.

Keywords
Mobilization of PBSC; RICE/G-CSF; Plerixafor; ASCT

1. Introduction

Patients with advanced non-Hodgkin's lymphoma (NHL) can be successfully treated with high-dose therapy followed by infusion of autologous peripheral blood stem cells (ASCT). The reported disease-free survival (DFS) rates for patients with diffuse NHL in second complete remission or not in remission at time of ASCT, for patients with mantle cell NHL, and for patients with transformed follicular NHL are 38% at three years, 28% at three years, 39% at five years, and 49% at four years, respectively [1-3].

Autologous peripheral blood stem cells (PBSCs) are routinely measured by fluorescence-activated cell sorting (FACS) analysis for CD34+ cells [4]. PBSCs are known to be increased in the peripheral blood by treatment with granulocyte-colony stimulating factor (G-CSF) before collection of PBSCs by apheresis [5, 6]. PBSCs in NHL patients are routinely collected off either growth factor support alone or after chemotherapy and growth factor.

The successful engraftment of PBSCs post ASCT is well correlated with the number of CD34 cells infused in the stem cell product [7]. The optimum number of CD34 cells utilized for transplantation varies between institutions, but is suggested to be 5×10^6 CD34 cells/kg, with 2×10^6 CD34 cells/kg being the minimum number required. However, the infusion of 5×10^6 CD34 cells/kg results in faster engraftment and improved survival [8, 9]. The success rate of engraftment after autologous PBSC transplantation is ≥90% [8].

Patients are considered "poor mobilizers" if they do not collect 2×10^6 CD34 cells/kg in a certain number of days of apheresis (e.g., 5 days or less) [8, 10, 11]. In the literature, up to 20% of NHL patients are reported to have a hard time initially mobilizing PBSC [8, 10-12]. Consequently, this may result in multiple attempts to collect stem cells with increased costs.

We had looked previously at our center at 370 lymphoma patients in which we attempted to mobilize autologous PBSC without using Plerixafor. The median age was 52 years old. Median number of previous regimens was two. Overall, 69% had persistent disease in their pre-transplant evaluation. Only 10% of the patients were mobilized with growth factor (G-CSF) alone. The factors associated with increased number of days of apheresis were previous fludarabine therapy (estimate effect (EE) 0.5, 95% confidence interval (CI) -0.03-1), and mobilization with G-CSF alone, EE 0.7, (95% CI. 0.24-1.12). What correlated with a decreasing number of CD34 cells/kg collected on the first day of apheresis was age, (EE 0.1, 95% CI 0.03-0.96), number of cycles of Hyper-CVAD previously received (EE 0.5, 95% CI 0.02-0.96), previous fludarabine therapy, (EE 3.4, 95% CI 0.11-6.63) and mobilization with G-CSF alone, (EE 3.9, 95% CI 1.1-6.74). What correlated with overall decreased total number of CD34 cells/kg collected was age (EE 0.1, 95% CI 0.06-0.17), number of cycles of Hyper-CVAD previously received (EE 0.5, 95% CI 0.12-0.93), previous fludarabine therapy (EE 2.8, 95% CI 0.04-5.6) and mobilization with G-CSF alone (EE 4.1, 95% CI 1.7-6.45). In these patients, we

collected a median of 3.7 × 10^6 CD34 cells/kg and 5% of all of our lymphoma patients failed to mobilize on the initial attempt but subsequently were re-challenged and were able to get overall an adequate total number of PBSC on further recollection attempts. Factors associated with neutrophil engraftment post ASCT was only a history of previous radiation therapy (EE -1.2, 95% CI -2--0.04), and for platelet engraftment was previous therapy with fludarabine (EE 3.4, 95% CI 0.7-6.1), number of cycles of Hyper-CVAD previously received (EE 0.6, 95% CI 0.2-1.0) and total number of CD34 cells/kg infused (EE -0.25, 95% CI -0.4--0.05). Mobilization off of RICE and G-CSF did not impact statistically in our univariate analysis models for any of these factors above that we assessed.

Hematopoietic stem cells express on their surface the cognate receptor chemokine (C-X-C motif) receptor 4 (CXCR4) that attracts them to and helps them to anchor to BM stromal cell surface stromal cell derived factor -1α (SDF-1α) [13]. Plerixafor is a selective, reversible inhibitor of the binding of SDF-1α to CXCR4. Plerixafor-induced elevations in circulating hematopoietic progenitor cell levels result from the disruption of the chemo attractant and cell adhesion effects. Plerixafor, given with G-CSF, is known to increase the number of circulating blood CD34 cells and results in a higher number of CD34 cells being collected during apheresis [14]. When Plerixafor (160 or 240 micrograms/kg) was given after 4 days of G-CSF (10 micrograms/kg/day) to NHL patients, apheresis yields were significantly better compared to when the patients were given G-CSF alone [15]. PBSC collection with plerixafor and G-CSF thus has a major benefit by increasing the circulating number of PBSC and consequently decreasing the number of apheresis sessions required to collect a sufficient number of PBSCs for ASCT. Plerixafor and G-CSF also provide a level of predictability for the successful timing of apheresis collection, resulting in better optimizing of clinical resources.

Many patients with NHL, though, need to receive salvage chemotherapy for tumor debulking to make them better candidates for ASCT. RICE (rituximab, ifosfamide, carboplatin, and etoposide) is a standard debulking therapy that has been routinely used to control disease. RICE and G-CSF can also be used as in vivo purging to collect a stem cell product without tumor contamination. As a result, we felt that it would be more efficient to collect autologous PBSC off RICE, G-CSF and Plerixafor to increase the CD34 cell product yield and to minimize the number of apheresis sessions. In this paper, we report on the results of our study in using RICE/G-CSF and Plerixafor to mobilize autologous PBSC. Also, since relapse remains a problem after ASCT for NHL and to date the characterization of lymphocyte subpopulations mobilized with G-CSF and Plerixafor is poorly studied, we do also descriptively report on the immune content of the product collected in our study.

2. Materials and Methods

2.1 Patients

After giving informed consent, twenty patients were treated on the Fred Hutchinson Cancer Center IRB-approved protocol.

Inclusions criteria for getting study therapy included: diagnosis of CD20+ NHL, ≥18 years old, having adequate organ function as measured by: left ventricular ejection fraction at rest ≥50%, bilirubin ≤2.0 mg/dL (except for isolated hyperbilirubinemia attributed to Gilbert syndrome), ALT and AST ≤3× the upper limit of normal (ULN) and creatinine clearance >50 mL/min. Patient needed to be planned to undergo ASCT within 3 months after collection of PBSCs.

Exclusion criteria included: Karnofsky performance score <70%, uncontrolled bacterial, viral, or fungal infection (defined as currently taking medication with progression or no clinical improvement), pregnant or breastfeeding, unwilling to use contraceptive techniques, had prior autologous or allogeneic transplant, was HIV positive or was hepatitis B carrier. Patients could not have been treated on another investigational therapy within 4 weeks of enrolling on our study. Patients were also deemed ineligible for therapy if they had history of a prior other malignancies, with exception of resected basal cell carcinoma or treated cervical carcinoma or breast cancer in situ. Patients with previous history of cancer that treated with curative intent >5 years were allowed on the study. There was nothing stipulated in the exclusion criteria for the percent of BM lymphoma involvement that would prevent enrollment on the study.

See Table 1 for characteristics of patients.

Table 1 Patients Characteristics.

Characteristic	**Patients (n = 20)**
Sex	
Male	14 (30%)
Female	6 (70%)
Median Number of cycles	8 (range 3-15)
History of previous radiation therapy	4 (20%)
History of previous Rituxan pre-RICE	20 (100%)
Number of regimens before RICE	
One regimen	2 (10%)
Two-three regimens	10 (50%)
≥ Four regimens (range 4-8)	8 (40%)
Median Age, years	66 (range: 42-72) years
# patients ≥65 years	12 (60%)
# patients ≥70 years	4 (20%)
Type of B cell NHL	
Follicular	2 (10%)
Transformed Diffuse	4 (20%)
Diffuse Large cell	7 (35%)
Mantle Cell	4 (20%)
Burkitt's/Richter's transformation from CLL	1/2 (15%)
Chemosenstivity at Time of Mobilization	
Sensitive	11 (55%)
Resistant	8 (40%)
Not determined	1 (5%)
Disease Status at Mobilization	
1st CR/2nd CR	2/2
Primary refractory	9
1st PR/2nd PR	4/2
1st relapse	1

2.2 Treatment Plan

See Table 2 for treatment schema.

Table 2 Treatment Plan.

Days	Drugs	Doses*
1	Rituxan	375 mg/m^2 IV
2	Etoposide	100 mg/m^2 IV
3	Etoposide	100 mg/m^2 IV
3	Carboplatin	AUC = 5 (calvert formula, maximum 800 mg IV
3	Ifosfamide	5 g/m^2 CIV over 24 hours
3	Mesna	5 g/m^2 CIV over 24 hours
4	Etoposide	100 mg/m^2 IV
6 to complete collections	G-CSF (non-pegylated)	10 mcg/kg sc

*M^2 based on actual weight

Patients received hydration, anti-emetics, standard infectious surveillance and prophylactic antibiotics per institutional guidelines. Of note, patients were also prescribed Imodium prn for diarrhea to use after starting Plerixafor. There was no dose reduction of therapy allowed.

Twenty-four hours after recovery of counts from their nadir, CD34 blood cell counts were started to be tested daily in the morning. When the CD34 blood count was ≥5 cells/microliter, Plerixafor 240 microgram/kg SC, rounded to nearest 2 mg, was added that evening, at most 10 to 14 hours prior to the planned initiation of apheresis. Plerixafor was daily continued until completion of collection, with plan for a maximum of 4 doses. G-CSF was administered in the morning approximately 1 hour prior to each daily apheresis. Plerixafor and G-CSF were administered as SC injections at separate sites. All apheresis was done as large volume per standard operating procedure at our institution. If clinically indicated, the dose of G-CSF could be increased at attending physician's discretion. All G-CSF and Plerixafor were stopped if WBC reached ≥90,000. Recommended goal of collection was ≥5 × 10^6 CD34 cells/kg, with a minimum collection goal of 2.5 × 10^6 CD34 cells/kg over 4 days acceptable.

The Plerixafor dose calculation was based on the patient's weight at screening pre therapy. The dose of Plerixafor was adjusted for patients who weigh >30% over their IBW and then we used adjusted body weight (ABW = IBW + 0.4 (actual weight - IBW). Otherwise, the dose was based on actual body weight. G-CSF dosing was based on actual weight unless >125% of ideal weight then dosing was based on adjusted weight per institutional guidelines.

2.3 Lab Testing

Six color flow cytometry staining was done for assessing for tumor contamination in the product and flow cytometry was done for assessing immune cell subpopulations [16]. NK and LAK activity were measured by a standard 4 hour ^{51}Cr-release assay using K562 and Daudi as target cells. Briefly, 1-2 × 10^6 target cells (K562 or Daudi) in 500 µl of CRA medium were labeled with 50-150 uCi Na_2 $^{51}CrO_4$ (New England Nuclear, Dupont, Boston, MA) at 37°C in 5% CO_2 in humidified air for one hour. After labeling, the cells were washed four times with warm medium before being re-suspended at

a concentration of 5×10^4 cells/ml. Five $\times 10^3$ target cells were added to the U-bottom wells of a 96 well tissue culture plate (Falcon). The NK-sensitive tumor cell line, K562, was used as a target for NK activity and lysis of the NK-insensitive tumor cell line, Daudi, was used as a measurement of LAK activity. Effector cells in 100 µl of CRA medium were then added to the wells at various effector-to-target ratios (E/T). After addition of the effector cells, every plate was centrifuged at room temperature for ten seconds at 1000 rpm. After incubation at 37°C 5% CO_2 in humidified air for four hours, the plates were centrifuged for 2 minutes at 1000 rpm. Then, 100 µls of supernatant was removed from each well and the amount of radioactivity counted in a gamma counter. Triplicate wells were assayed for each E/T. Spontaneous release was determined by evaluating the supernatant from tumor targets not incubated with effector cells. Spontaneous release was <10% per hour. Total release was determined by lysing the tumor targets with 1% NP40 detergent. The percentage cytotoxicity at a given E/T was calculated according to the following formula:

$$\%\ \text{Specific Lysis} = 100 \times \frac{(\text{Test samples CPM} - \text{Medium control CPM})}{(\text{Detergent control CPM} - \text{Medium control CPM})}$$

Lytic units were determined as the number of effector cells required to cause 20% lysis of 5000 target cells.

2.4 Statistical Analysis

As we expected with chemotherapy to have hematological toxicity, they were not graded. Other toxicity though was graded by the NCI's Common Terminology Criteria for Adverse Events (CTCAE), version 3. The probabilities of OS and PFS were estimated from the date of study therapy to the date of death or the date of disease progression. Data were censored at date of last contact according to Kaplan and Meier [17].

3. Results

3.1 Patients' Characteristics

As outlined in Table 1, the patients treated on our study were older individuals and heavily treated before receiving mobilization therapy. Overall, 40% had received $\geq$ four different previous chemotherapy regimens (range 4-8). They also were older as median age was 66 years, with 60% ≥65 years and 20% ≥70 years old. The majority also had diffuse large cell NHL but 3 patients had diagnosis of Burkitt's or Richter transformation from CLL. Forty-five percent of the patients had primary refractory NHL and 40% had chemo-resistant disease when they received RICE/G and Plerixafor for mobilization.

3.2 Toxicity

No patients died of complications of the study therapy. No one met the stopping rules of the study for significant toxicity. Hematological toxicities are not reported for the study as all patients were expected to get low counts due to chemotherapy. See Table 3 for number of episodes of non-hematological, non-infectious grade 3-4 toxicity with RICE and G-CSF and Plerixafor. Table 3 also shows the number of episodes of any toxicity seen after Plerixafor was added. As expected, diarrhea

grade 1-2 was most common side effect associated with the addition of Plerixafor. Of note, since patients were given Imodium prior to use after starting Plerixafor for diarrhea symptoms, this use of Imodium may have impacted on severity of grade and episodes of diarrhea reported by the patients. See Table 3 for number of infection episodes seen in all study patients. Of note, no patient's PBSC product was contaminated with bacteria on sterility testing.

Table 3 Toxicities.

Number of Episodes Non-Infections Non-Hematological Toxicities ≥ Grade 3		
Category	**Grade 3**	**Grade 4**
Febrile neutropenia	1	0
Asymptomatic line-associated DVT	1	0
Hyperglycemia	1	0
Number of Episodes of Non-Infections Non-Hematological Toxicities Seen After Added Plerixafor		
Category	**Grade 1**	**Grade 2**
Diarrhea	3	2
Injection site irritation	1	0
Number of Episodes of Infections		
Type		
C difficile colitis	1	
Aspiration pneumonia	1	
Fever (no organism identified)	2	
Cellulitis	1	
Upper respiratory tract infection	1	

3.3 Response to Therapy

Three patients were taken off study therapy by their attending physicians as CD34 blood count was <5 cells/μL and did not get Plerixafor as per study. All of these patients collected after additional therapy, including Plerixafor and G-CSF. The number of days of collections of these three patients was 2, 3 and 5 days, respectively. The median number of cells collected was 4.44×10^6 CD34 cells/kg (range 2.76-4.58).

In the remaining 17 patients collected per study therapy, the apheresis product was flowed for contaminating NHL cells and 15 out of 17 were flow negative. Two products were flow positive: one with history of Richter transformed from CLL and second with history of transformed follicular. The overall increase in CD34 blood counts from blood checked day before Plerixafor was added to day after was 2.6-9 fold increase. In the 17 patients, 15 (88%) out of 17 required only one day of collection. Two patients required two days of collections to reach the collection goal. The number of CD34 cells collected was 12.92×10^6 CD34 cells/kg (range 5.44-83.76).

One patient got treated with RICE/G and Plerixafor and did not go to transplant due to disease progression. Thus, sixteen patients underwent transplant. The median number of CD34 cells/kg infused were 7.29×10^6 (range 4.63-28.10). The median day to reaching ANC ≥500 was 12 days (range 10-19) in absence of G-CSF support and platelets ≥20K was 11 days (range 0-19) and platelets

count ≥50K was 16.5 days range 11-42 days. There was no primary or secondary graft failures post ASCT.

Seven (41%) out of 17 patients have now relapsed NHL disease. Ten out of 17 (58%) are still in remission, including one patient though with history of Waldenström's who has now had reoccurrence of Waldenström's but has no relapse of the diffuse large cell NHL for which they underwent the ASCT. Eleven patients out of the 17 who completed study therapy are alive (65%). There have been 6 deaths, from NHL (n = 5) and from ARDS/heart attack and infection (n = 1). Median follow-up is 4.17 years (range 3.7-6.69 years).

3.4 Descriptive Lab Correlative Studies

With respect to the apheresis PBSC product, see Table 4. Only two patients had NK activity in their PBSC product and six patients had LAK activity. NK activity was the most recovered lytic activity seen in day +28 blood post TX (n = 14). There was no correlation between lytic activity in apheresis product and day +28 blood expression post TX. Table 5 shows the number of lymphocytes subsets per 100,000 cells in collected apheresis product and day + 28 post ASCT. Only three patients had absolute lymphocyte count >500 on day 14 post TX, with a median absolute lymphocyte count (ALC) of 295 (range 180-1,160). All patients, though, did have an ALC ≥500 by day 28, with a median of 1170 (range 680-5510). There was no significant correlation between apheresis product with the number of immune cells seen in blood on day +28 post-ASCT or with NHL relapse or remission status.

Table 4 LAK (Daudi) and NK (K562) Lytic Activity*.

PBSC product	Day + 28 post ASCT blood
+D -NK, n = 4	-D +NK, n = 3; +D +NK, n = 1
-D -NK, n = 10	-D +NK, n = 7, -D -NK, n = 2 and +D +NK, n = 1
+D +NK, n = 2	-D +NK, n = 2

*Positive (+) expression of killing is >20% lytic activity over control and negative (-) is <20% lytic activity over control.

Table 5 Lymphocyte Populations in Apheresis Stem Cell Product and Day +28 Blood Post ASCT.

PBSC product		Day + 28 blood post ASCT
Gated on	Median number of cells/100,000 cells (range)	Median number of cells/100,000 cells (range)
Gated on 3+		
CD4+	61,450 (11,000-75,400)	37,150 (18,100-70,500)
CD4+/8+	1900 (700-5070)	1500 (300-5,400)
CD8+	35,000 (28,000-86,100)	61,000 (27,500-75,400)
Gated on 3-		
CD20+	390 (40-11,140)	500 (10-3,900)
CD56+16+	5600 (510-34,200)	38,050 (1,370-78,700)

	(CD69+ subpopulation: 2.73% (0.8-98.2%)	(CD69+ subpopulation 1.93% (0.39-5.91%)
Gated on CD4+		
Fox P+25+	0 (0-29,300)	0 (0-200)
CD127 low and 25+	2500 (800-29,700)	11,1000 (480-30,600)

4. Discussion

The best way to mobilize autologous PBSC remains controversial. Many prefer the convenience of growth factor alone. But historically, chemotherapy and growth factor have been more successful at collecting higher number of stem cells at a price of increased toxicity and higher resource utilization [18]. RICE therapy remains the major chemo-mobilization regimen used for lymphoma control and mobilization of autologous PBSC in CD20+ lymphoma patients.

Little has been published, though, on the efficacy and safety of comparing RICE with G-CSF vs Plerixafor growth factor alone regimens. Dhakal et al showed that chemo-mobilization with ICE was superior to routine Plerixafor use with G-CSF or just in time approach for use of Plerixafor [19]. The median collected apheresis product was 5.35×10^6 CD34 cells/kg for chemotherapy vs routine Plerixafor 3.15×10^6 CD34 cells/kg and just in time Plerixafor 3.6×10^6 CD34 cells/kg, $p > 0.001$. There was no significant difference in the number of apheresis sessions, with a median of two sessions in each group. Median time to ANC and platelet engraftment post ASCT was faster with chemoembolization product 10.3 vs. 12.1 vs. 11.6 days for ANC and for platelets 13.7 vs. 20.3 vs. 17.1, respectively for chemo-mobilization, Plerixafor routine and just in time Plerixafor. The differences in days to engraftment most likely were due to differences in the median number of CD34 cells/kg infused in the groups.

Recently, Jagirdar et al reported on the addition of Plerixafor to chemotherapy, including twenty lymphoma patients who got RICE therapy and seventeen patients who got cyclophosphamide [20]. Plerixafor was added after the ANC recovered to 1.5×10^9 cells/L. In their study, 33% of MM or lymphoma patients required more than one day of apheresis and Plerixafor treated patients collected a total of 7.8×10^6 CD34 cells/kg.

In lymphoma patients in order to offer the curative therapy of an ASCT, an adequate number of autologous PBSC need to be collected. Historically, 10-30% of patients are not able to collect a minimum number of $>2 \times 10^6$ CD34 cells/kg and previous therapy with bendamustine, fludarabine and Hyper-CVAD as well as age have all contributed to this failure [21-23].

In our paper, we presented data on using RICE and G-CSF and Plerixafor for treating CD 20+ NHL patients. The median number of cells collected was 12.92×10^6 CD34 cells/kg and higher than that reported in Jagirdar and Dhakal et al studies [19, 20]. Toxicity in our study included 5 episodes of diarrhea (grades 1-2) and one episode of site injection irritation (grade 1) after Plerixafor was added. Collection was completed in one apheresis session in 15 (88%) out of 17 patients treated per study. It is hard to comment on the three patients taken off our study by their attending physician as they did not meet the criteria to be removed from the study and in fact might have been able to collect if CD34 blood cells had been checked again in a couple of days, especially if G-CSF dose had been increased as allowed on the study. With a median of 7.29×10^6 CD34 cells/kg infused in our patients, the median time to ANC engraftment was 12 days (in the absence of post TX G-CSF supportive care) and the median day to Platelet ≥20K was 11 days and ≥50K was 16.5 days, respectively. Engraftment

was what would have expected for the number of CD34 cells/kg infused. There were no primary or secondary graft failures on our study. It should also be pointed out that we used a CD34 blood cell count of equal or >5 cells/μL to add Plerixafor and that a CD 34 blood count of 5 is lower than most use in their real time formula for adding Plerixafor. The patients on our study also were an older population, with a median age of 66 years old and 12 (60%) of the patient were ≥65 years old and 4 (20%) were ≥70 years old. We and others have shown that age is an important factor in determining ability to get a good stem cell product. Our patients were also heavily treated, with 90% of them having a history of two or more regimens before getting study therapy and 40% of them with a history of ≥4 regimens. Given the small number of patients it was hard for us to comment on the effect of getting previously fludarabine, bendamustine or four or more cycles of Hyper-CVAD and also take into account the age of the patient and number of different regimens. Indeed, for the NHL patient, it is easier if they are getting chemotherapy for debulking therapy to take advantage of the therapy and use our study therapy approach as the majority collected in only one day.

Limitations of our study though, include the small number of patients treated on the study, and variability in previously received treatments and different NHL pathology included in the study. In addition, given the number of cells collected, it is not obvious that all patients really needed Plerixafor and many would have not received it under our real-time risk adapted rescue strategy [24]. Another limitation is the lack of control for intra-physician variability in decision making about how to treat patients with low blood CD34 cell counts after salvage chemotherapy and G-CSF as show with 3 patients coming off study at the discretion of attending physician caring for them before G dose was increased as recommended in the study. Finally, our approach to how to manage these patients today would be different given our recent publication [25].

5. Conclusions

In summary, the combination of Plerixafor to RICE/G-CSF overall was well tolerated, was highly effective in mobilizing autologous PBSC and allowed collection in one day of apheresis in 88% of our elderly and heavily treated NHL patient population.

Acknowledgments

Funding for the study was provided by Sanofi/Genzyme. The authors wish to thank Julia Montes for her help and support as study staff.

Author Contributions

LAH was the principal investigator, designed the study, analyzed the data, recruited patients and wrote the manuscript. LCS and ML oversaw the apheresis and cryopreservation of the autologous PBSC. All authors reviewed the manuscript for intellectual content and approved the final version. Sanofi/Genzyme provided funding for the study.

Funding

Dr. Holmberg received funding for studies from Millennium-Takeda, Sanofi/Genzyme, Seattle Genetics and Merck Sharp & Dohme, Inc. and royalty from UpToDate. She is also a consultant for NCCN and Jazz Pharmaceuticals.

Competing Interests

The other authors have declared that no competing interests exist.

References

1. Vose JM, Rizzo DJ, Tao-Wu J, Armitage JO, Bashey A, Burns LJ, et al. Autologous transplantation for diffuse aggressive non-Hodgkin lymphoma in first relapse or second remission. Biol Blood Marrow Transplant. 2004; 10: 116-127.
2. Cao TM, Horning S, Negrin RS, Hu WW, Johnston LJ, Taylor TL, et al. High-dose therapy and autologous hematopoietic-cell transplantation for follicular lymphoma beyond first remission: The Stanford University experience. Biol Blood Marrow Transplant. 2001; 7: 294-301.
3. Ganti AK, Bierman PJ, Lynch JC, Bociek RG, Vose JM, Armitage JO. Hematopoietic stem cell transplantation in mantle cell lymphoma. Ann Oncol. 2005; 16: 618-624.
4. Croop JM, Cooper R, Seshadri R, Fernandez C, Graves V, Kreissman S, et al. Large-scale mobilization and isolation of CD34+ cells from normal donors. Bone Marrow Transplant. 2000; 26: 1271-1279.
5. van Der Auwera P, Platzer E, Xu ZX, Schulz R, Feugeas O, Capdeville R, et al. Pharmacodynamics and pharmacokinetics of single doses of subcutaneous pegylated human G-CSF mutant (Ro 25-8315) in healthy volunteers: Comparison with single and multiple daily doses of filgrastim. Am J Hematol. 2001; 66: 245-251.
6. Cassens U, Momkvist PH, Zuehlsdorf M, Mohr M, Kienast J, Berdel WE, et al. Kinetics of standardized large volume leukapheresis (LVL) in patients do not show a recruitment phenomenon of peripheral blood progenitor cells (PBPC). Bone Marrow Transplant. 2001; 28: 13-20.
7. Allan DS, Keeney M, Howson-Jan K, Popma J, Weir K, Bhatia M, et al. Number of viable CD34(+) cells reinfused predicts engraftment in autologous hematopoietic stem cell transplantation. Bone Marrow Transplant. 2002; 29: 967-972.
8. Gazitt Y, Freytes CO, Callander N, Tsai TW, Alsina M, Anderson J, et al. Successful PBSC mobilization with high-dose G-CSF for patients failing a first round of mobilization. J Hematother. 1999; 8: 173-183.
9. Blystad AK, Delabie J, Kvaloy S, Holte H, Valerhaugen H, Ikonomou I, et al. Infused CD34 cell dose, but not tumour cell content of peripheral blood progenitor cell grafts, predicts clinical outcome in patients with diffuse large B-cell lymphoma and follicular lymphoma grade 3 treated with high-dose therapy. Br J Haematol. 2004; 125: 605-612.
10. Narayanasami U, Kanteti R, Morelli J, Klekar A, Al-Olama A, Keating C, et al. Randomized trial of filgrastim versus chemotherapy and filgrastim mobilization of hematopoietic progenitor cells for rescue in autologous transplantation. Blood. 2001; 98: 2059-2064.
11. Morris T, Ranaghan L, Drake M. Poor stem cell mobilizers--variation in response to G-CSF dose escalation. Bone Marrow Transplant. 1999; 24: 448-449.
12. Stockerl-Goldstein KE, Reddy SA, Horning SF, Blume KG, Chao NF, Hu WW, et al. Favorable treatment outcome in non-Hodgkin's lymphoma patients with "poor" mobilization of peripheral blood progenitor cells. Biol Blood Marrow Transplant. 2000; 6: 506-512.

13. Broxmeyer HE, Kim CH, Cooper SH, Hangoc G, Hromas R, Pelus LM. Effects of CC, CXC, C, and CX3C chemokines on proliferation of myeloid progenitor cells, and insights into SDF-1-induced chemotaxis of progenitors. Ann N Y Acad Sci. 1999; 872: 142-162.
14. Hubel K, Liles WC, Broxmeyer HE, Rodger E, Wood B, Cooper S, et al. Leukocytosis and mobilization of CD34+ hematopoietic progenitor cells by AMD3100, a CXCR4 antagonist. Support Cancer Ther. 2004; 1: 165-172.
15. DiPersio JF, Micallef IN, Stiff PJ, Bolwell BJ, Maziarz RT, Jacobsen E, et al. Phase III prospective randomized double-blind placebo-controlled trial of plerixafor plus granulocyte colony-stimulating factor compared with placebo plus granulocyte colony-stimulating factor for autologous stem-cell mobilization and transplantation for patients with non-Hodgkin's lymphoma. J Clin Oncol. 2009; 27: 4767-4773.
16. Wu D, Wood BL, Fromm JR. Flow cytometry for non-Hodgkin and classical Hodgkin lymphoma. In: Lymphoma. Methods in molecular biology. Totowa: Humana Press; 2013. pp. 27-47.
17. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc. 1958; 53: 457-481.
18. Giralt S, Costa L, Schriber J, Dipersio J, Maziarz R, McCarty J, et al. Optimizing autologous stem cell mobilization strategies to improve patient outcomes: Consensus guidelines and recommendations. Biol Blood Marrow Transplant. 2014; 20: 295-308.
19. Dhakal B, Veltri LW, Fenske TS, Eastwood D, Craig MD, Cumpston A, et al. Hematopoietic progenitor cell mobilization with ifosfamide, carboplatin, and etoposide chemotherapy versus plerixafor-based strategies in patients with Hodgkin and non-Hodgkin lymphoma. Biol Blood Marrow Transplant. 2016; 22: 1773-1780.
20. Jagirdar N, Harvey RD, Nooka A, Flowers C, Kaufman J, Lonial S, et al. Plerixafor in combination with granulocyte-colony-stimulating factor after chemotherapy increases mobilization efficiency in patients with lymphoma or myeloma: Results of a Phase II clinical trial. Transfusion. 2015; 55: 2351-2357.
21. Kuittinen T, Nousiainen T, Halonen P, Mahlamaki E, Jantunen E. Prediction of mobilisation failure in patients with non-Hodgkin's lymphoma. Bone Marrow Transplant. 2004; 33: 907-912.
22. Gordan LN, Sugrue MW, Lynch JW, Williams KD, Khan SA, Wingard JR, et al. Poor mobilization of peripheral blood stem cells is a risk factor for worse outcome in lymphoma patients undergoing autologous stem cell transplantation. Leuk Lymphoma. 2003; 44: 815-820.
23. Akhtar S, Tbakhi A, Humaidan H, El Weshi A, Rahal M, Maghfoor I. ESHAP + fixed dose G-CSF as autologous peripheral blood stem cell mobilization regimen in patients with relapsed or refractory diffuse large cell and Hodgkin's lymphoma: A single institution result of 127 patients. Bone Marrow Transplant. 2006; 37: 277-282.
24. Gopal AK, Karami M, Mayor J, Macebeo M, Linenberger M, Bensinger WI, et al. The effective use of plerixafor as a real-time rescue strategy for patients poorly mobilizing autologous CD34(+) cells. J Clin Apher. 2012; 27: 81-87.
25. Holmberg LA, Linenberger M, Connelly-Smith L. Successful mobilization of autologous hematopoietic peripheral blood stem cells after salvage chemotherapy in patients with low CD34 blood cell counts. Transplant Cell Ther. 2022; 28: 754-759.