Original Study

Predictors of CD34$^+$ Cell Mobilization and Collection in Adult Men With Germ Cell Tumors: Implications for the Salvage Treatment Strategy

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Abstract

Tandem or triple high-dose chemotherapy (HDCT) cycles with CD34$^+$ cell support is an increasingly used option in the salvage therapy of male germ cell tumors (GCT). We analyzed the combined series of 2 institutions with the aim to optimize the salvage strategy by identifying potential limitations in administering HDCT. The negative effect of previous chemotherapy load on the CD34$^+$ cell mobilization and harvest was observed. This might have implications in salvage treatment strategy.

Background: High-dose chemotherapy with tandem or triple carboplatin and etoposide course is currently the first curative choice for relapsing GCT. The collection of an adequate amount of hematopoietic (CD34$^+$) stem cells is a priority. **Patients and Methods:** We analyzed data of patients who underwent HDCT at 2 referral institutions. Chemotherapy followed by myeloid growth factors was applied in all cases. Uni- and multivariable models were used to evaluate the association between 2 prespecified variables and mobilization parameters. Analyses included only the first mobilizing course of chemotherapy and mobilization failures. **Results:** A total of 116 consecutive patients underwent a mobilization attempt from December 1995 to November 2012. Mobilizing regimens included cyclophosphamide (CTX) 7 gr/m^2 (n = 39), cisplatin, etoposide, and ifosfamide (PEI) (n = 42), paclitaxel, cisplatin, and gemcitabine (TPG) (n = 11), and mixed regimens (n = 24). Thirty-seven percent were treated in first-line, 50% (n = 58) in second-line, 9.5% (n = 11) and 3.4% (n = 4) in third- and fourth-line settings, respectively. Six patients did not undergo HDCT because they were poor mobilizers, 2 in first- and second-line (1.9%), and 4 beyond the second-line (26.7%). In the multivariable model, third-line or later setting was associated with a lower CD34$^+$ cell peak/μL ($P = .028$) and a lower total CD34$^+$/kg collected ($P = .008$). The latter was also influenced by the type of mobilizing regimen ($P < .001$).

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Conclusion: A decline in significant mobilization parameters was found, primarily depending on the pretreatment load. Results lend support to the role of $CD34^+$ cell mobilization in the therapeutic algorithm of relapsing GCT, for whom multiple HDCT courses are still an option, and potentially a cure.

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Introduction

A widely recognized option in the salvage setting of germ cell tumors (GCT) is represented by a dose intensification strategy with the use of autologous hematopoietic stem cell progenitors (herein referred to as $CD34^+$ cells or alternatively, peripheral blood stem cells [PBSC]).[1-4] The renaissance of this approach was because of the long-term responses and progression-free survival estimates provided in patients undergoing double or triple courses with high-dose (HD) carboplatin and etoposide as first or later salvage treatment.[4,5] A proportion of relapsing or refractory patients exceeding 70% in the second-line and 50% later actually achieved a cure, although this apparent superiority over conventional-dose regimens is still hampered by the lack of a randomized comparison and the retrospective quality of results in most cases. In the past years investigators have focused on identifying prognostic factors for survival of patients relapsing to cisplatin, etoposide, and bleomycin (PEB), with the result of establishing a prognostic model based on 5 categories (from very low risk to very high risk).[5] These criteria were relative to patient and disease characteristics and to the timing of relapse, but there is a paucity of data concerning factors that affect the key issue of the collection of PBSC in these patients. Available information dates back to approximately 15 years ago, since the publication of the predictive role of preleukapheresis cell counts on collection results and the dose of PBSC transplanted in association with engraftment of patients with GCT.[6] Since then, no further articles reported on this issue, and the potential benefit of high-dose chemotherapy (HDCT), particularly in the subset of patients in whom multiple chemotherapy regimens have failed (namely, the third-line setting) or who have refractory disease, has become of noteworthy importance. Hence, there is the need to provide updated guidance for PBSC mobilization and collection in men with GCT, particularly by framing the issue into the concerns of modern therapeutic strategies. The unique availability of multiple cohorts of patients who mobilized in different treatment settings allowed us to evaluate and compare major clinical determinants of mobilization and engraftment in the disease and treatment course.

Patients and Methods

We reviewed the premobilization characteristics of patients, and treatments and mobilization parameters of GCT patients who were candidates for transplantation. All those who underwent at least 1 mobilization attempt, including mobilization failures, were analyzed.

Patient Population and Mobilization Procedure

Candidate adult men with GCT were referred to Fondazione IRCCS Istituto Nazionale dei Tumori (INT), Milan, Italy (n = 99) and Fondazione IRCCS Policlinico San Matteo, Pavia, Italy (n = 17) for hematopoietic stem cell collection and subsequent HD chemotherapy. All patients mobilized with the use of chemotherapy and myeloid growth factors. Granulocyte colony-stimulating factor (G-CSF) was administered in all cases; 8 patients also received granulocyte-macrophage colony-stimulating factor 500 $\mu g/m^2/d$, and 4 poor mobilizers received additional plerixafor 240 μg/kg subcutaneously before apheresis. The PBSC count was measured beginning with day 10 of chemotherapy and then daily, until the end of collection. The methods for enumerating PBSC changed over time from a double-platform to a single-platform flow cytometry.[7,8] The latter used CD34-phycoerythrin and CD45-fluorescin isothiocyanate monoclonal antibodies, TruCount fluorospheres (BD Biosciences) in combination with the viability dye, 7-amino actinomicyn D, to exclude dead cells, and the International Society for Hematotherapy and Gragt Engineering gating strategy, as previously reported.[8] The peak of $CD34^+$ cell count was defined as the maximum number of $CD34^+$ cells detected in the peripheral blood during mobilization. In all patients, PBSC collection was started the day after PBSC count of at least 10 cells/μL. The optimal target to undergo transplantation was $\geq 4.0 \times 10^6$/kg per planned transplant, and the minimum number required was 3.0×10^6/kg. PBSC collections were performed using a COBE Spectra cell separator (CaridianBTC).[9]

Mobilization and Transplant Regimens

The choice of mobilization strategy and consequently the target number of $CD34^+$ cells depended on clinical recommendations and by the past and ongoing protocols in effect since 1995 at the 2 institutions.

Forty-three patients needed an amount of cells to undergo a single transplant. These were patients with poor prognosis GCT randomized from 1995 to 2007 to receive 4 cycles of PEB or a sequential HDCT in a multicentric Italian trial. This sequence included a single course of HD cyclophosphamide (CTX; eg, 7 gr/m^2) followed by PBSC harvest and 2 cycles of HD etoposide (1.2 gr/m^2 each) preceding the administration of a single HDCT course with HD-carboplatin area under the curve of 25 and stem-cell rescue.[10] All the other patients had to collect an amount of $CD34^+$ cells to undergo a double transplant. Twenty-nine of them entered an ongoing single-group, phase 2 trial with tandem HD-carboplatin (700 mg/m^2 days 1-3) and etoposide (750 mg/m^2 days 1-3) as first salvage treatment (registered with ClinicalTrials.gov, number NCT01172912). In this trial a single cycle of cisplatin, etoposide, and ifosfamide (PEI) was designed to collect PBSC. The remaining 44 patients gave informed consent to undergo the administration of tandem HD-carboplatin and

etoposide in the salvage setting (eg, second-line or beyond, except for 2 patients who received HD-etoposide only). Treatment was preceded by 1 or more mobilizing courses of conventional chemotherapy outside of a clinical trial. These protocols were sponsored and approved by the internal review board of Fondazione INT Milan, Italy.

Statistical Methods

Statistical analyses focused on the investigation of clinical parameters as possible predictors of adequate PBSC mobilization and harvest. Three indexes were taken as indicators of adequate PBSC mobilization: total number of $CD34^+$ cells collected, $CD34^+$ cell peak/μL, and time to $CD34^+$ cell peak since chemotherapy initiation. The identification of variables associated with engraftment kinetics was a secondary end point. The first mobilizing course/attempt was the objective of the uni- and multivariable analysis to overcome the bias of the different target numbers of $CD34^+$ cells to be harvested (eg, 1 or more planned transplants). We evaluated the association between the mobilization indexes and prespecified factors, such as chemotherapy regimen used for the mobilization attempt, and line of treatment. We selected the most frequently used regimens in each setting (eg, HD-CTX in first-line, PEI in second-line,[11] paclitaxel, cisplatin, and gemcitabine [TPG] in third-line[12,13]) and dichotomized the treatment setting between first-/second-line and third-line or beyond (ie, < 3 or ≥ 3). The association between the mobilization indexes and type or line of treatment was tested in a univariable setting using the Anderson and Darling test,[14] and multivariable analyses (MVA) were performed by applying quantile regression models[15] to test the association between the median value of mobilization indexes (log transformed) and type and line of treatment. The model results are presented as median ratio, corresponding 95% confidence interval, and likelihood ratio test *P* value.

Engraftment was defined as the number of days from PBSC reinfusion to recovery of absolute neutrophil count (ANC) greater than 500/μL, and transfusion independence for platelet count greater than 20,000/μL. Overall survival (OS) was calculated from date of treatment start to the date of death from any cause, or censored at the date of last follow-up for living patients. Univariable and multivariable Cox models were fitted; the covariates analyzed were: treatment setting (first- and second-line vs. third-line or beyond), $CD34^+$ cells reinfused (modeled as a continuous variable by means of a 3-knot restricted cubic spline),[16] and type of HDCT course (carboplatin-etoposide vs. other); the models accounted for clustering by patient.

The analyses were carried out using the SAS and R software (*http://www.r-project.org*). The results were considered statistically significant whenever a *P* value below 5% was achieved.

Results

In the time from December 1995 to November 2012, 116 patients were treated. Table 1 depicts the main patient and treatment characteristics. One hundred one patients were in either first- or second-line treatment, and 23 patients underwent more than 1 mobilization attempt. Overall, the median number of $CD34^+$ collected was 12.2×10^6/kg (interquartile range [IQR], 9.5 to 16.0) and median number of $CD34^+$ per leukapheresis was 5.8×10^6/kg (IQR, 2.7-10.0).

Table 2 shows the results of the univariable analysis, also represented in the box plots of Figure 1. In detail, median $CD34^+$ collected (primary end point) were 12.2×10^6 $CD34^+$/kg in first- or second-line (IQR, 9.4-16.2) and 8.1×10^6/kg beyond second-line ($P = .001$). The median number of $CD34^+$ cells collected were 10.6×10^6/kg (IQR, 9.2-15.0) with HD-CTX, 14.8 with PEI (IQR, 12.3-17.3), 10.0 with TPG (IQR, 8.2-14.3), and 8.5 with the remaining schedules (IQR, 3.5-12.1) (overall $P < .001$).

The peak of $CD34^+$ cells per μL was reached in a median of 13 days (IQR, 12-14) after HD-CTX, 16 days after PEI (IQR, 15-19), 10 days after TPG (IQR, 9-11), and 11 days for the remaining regimens. Median $CD34^+$ cells collected per leukapheresis were 7.7×10^6/kg (IQR, 4.6-11.9) in first- or second-line and 2.7×10^6/kg (IQR, 2.0-5.2) beyond the second-line ($P < .001$, not included in MVA). Major findings of the univariable analysis were confirmed in the multivariable model (Table 3) because the type of induction regimen was significantly associated with the time to $CD34^+$ peak ($P < .001$) and the total number of $CD34^+$ cells collected ($P < .001$), and the line of therapy was significantly associated with the $CD34^+$ peak/μL ($P = .028$) and the total number of $CD34^+$ cells collected ($P = .008$).

In analysis of only the 73 patients who were candidates for multiple transplants, 17 failed to collect the optimal $CD34^+$ cell target, 10/58 in second-line (17.2%) compared with 7/15 in third- or further line (46.6%; $P = .034$, using Fisher exact test). Notably, a total of 6 patients did not undergo HDCT because they failed to mobilize, 2 in first- and second-line (1.9%), and 4 beyond the second-line (26.7%). Three additional patients were rescued with off-label plerixafor. Eighty-seven patients received transplantation (75%) and the total number of HDCT courses was 131. Median number of $CD34^+$ cells reinfused was 6.8×10^6/kg (IQR, 5.4-8.6) per transplant according to the regimens provided in Table 1. Time to neutrophil (ANC) and platelet recovery was independent of the line of treatment (median always of 9 days) and there was no association between $CD34^+$ cells per kg reinfused and ANC or platelet recovery, documented using the Spearman correlation coefficient of -0.29 and -0.11, respectively. By stratifying patients according to whether they received less than ($n = 25$) or equal/more than 5.0×10^6 $CD34^+$ cells per kg ($n = 104$; 2 patients were not evaluable), time-to-engraftment cumulative distributions were overlapping between the 2 strata (data not shown).

In the univariable analysis for OS, conditioning regimen was significant (hazard ratio, 2.73; 95% confidence interval, 1.21-6.12; $P = .015$ at Wald test) and the line of treatment ($P = .086$) and the number of $CD34^+$ cells reinfused ($P = .255$) were not. Results were confirmed at the multivariable analysis (provided in Supplemental Table 1).

Discussion

In the landscape of increasingly used multiple HDCT courses in the salvage setting of GCT, the issue of collecting an adequate amount of hematopoietic stem cells was addressed with potential implication in the clinical practice. The nodal point is the importance of collecting and reinfusing a sufficient aliquot of $CD34^+$ cells, despite conflicting data on the predictive value of transfused $CD34^+$ cells for hematopoietic reconstitution. It was a standard policy in the present institutions to prioritize mobilization with

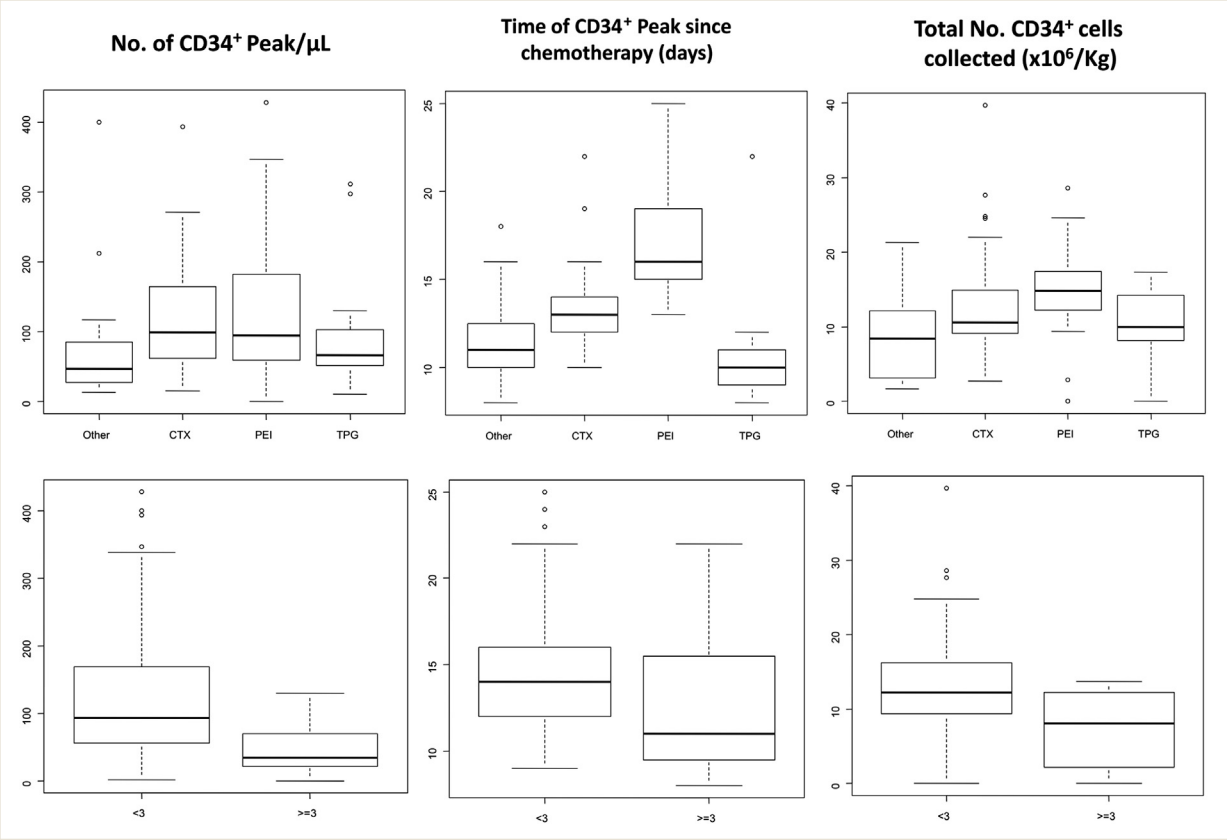
Table 1 Patient, Treatment, and Mobilization Characteristics

Characteristic	Patients, n	%	Attempts, n	%
Patients/Mobilization Attempts, n[a]	116	100	139	100
Target CD34$^+$ per kg per transplant	$\geq 4 \times 10^6$ per kg			
Age, Median (IQR), Years	31 (23-38)			
Time Frame	December 1995 to November 2012			
Treatment Setting				
First-Line	43	37.1	47	33.8
Second-Line	58	50.0	67	48.2
Third-Line	11	9.5	17	12.2
Fourth- or Fifth-Line	4	3.4	8	5.8
Mobilizing Chemotherapy				
CTX 7 g/m^2	39	33.7	39	28.1
PEI	42	36.2	43	30.9
T-I	12	10.3	19	13.7
TPG	11	9.5	17	12.2
Other	12	10.3	21	15.1
Growth Factors				
G-CSF	104	89.6	126	90.6
G-CSF with plerixafor	3	2.6	4	2.9
G-CSF with GM-CSF	8	6.9	8	5.8
G-CSF intravenously	1	0.9	1	0.7
Total Patients Undergoing HDCT	87	75		
Total Number of HDCT Courses	131			
Type of HDCT Course				
HD-CBDCA (AUC, 25)	33	25.2		
HD-CBDCA-VP16[b]	96	73.3		
HD-VP16	2	1.5		

Abbreviations: AUC = area under the curve; CBDCA = carboplatin; CTX = cyclophosphamide; G-CSF = granulocyte colony-stimulating factor; GM-CSF = granulocyte-macrophage colony-stimulating factor; HD = high-dose; HDCT = high-dose chemotherapy; IQR = interquartile range; PEI = cisplatin, etoposide, ifosfamide; T-I = paclitaxel, ifosfamide; TPG = paclitaxel, cisplatin, gemcitabine; VP16 = etoposide.
[a]Only the first mobilization course/attempt was included in the uni- and multivariable analysis (see the section, Statistical Analysis).
[b]CBDCA 700 mg/m^2 with VP16 750 mg/m^2 days 1-3.

chemotherapy plus G-CSF and to set the optimal target at 4.0×10^6 cells per kg for transplantation, according to our clinical experience and in agreement with other authors.[17-19] Yet, available findings for GCT and hematologic neoplasms recognized 2.5×10^6 cells per kg as a recommended cutoff to provide rapid hematopoietic recovery, and in the series of Indiana University a minimum of 1 million CD34$^+$ cells per kg was required for each cycle of HDCT.[3,6] The homogeneously high amount of CD34$^+$ cells transplanted is likely the reason we did not recognize any significant association with time to recovery, either analyzing the number of cells transplanted as a continuous variable or dichotomizing it as $<$ or $\geq 5.0 \times 10^6$/kg (data not shown). The primary objective of the present work was to frame the mobilization issue into the treatment options for GCT. Because the use of an HDCT strategy, basically entailing the administration of multiple courses of carboplatin and etoposide, might be referred to as the first option when feasible in the third-line setting (45% long-term responses), the issue of mobilization across lines and regimens might become of noteworthy importance. The peculiarity of this series was to include mobilizing attempts across each chemotherapy line and this lent us support to primarily analyze the effect of previous chemotherapy load by dichotomizing patients depending on the number of regimens already received. The uni- and multivariable analysis identified this variable as associated with the total number of CD34$^+$ cells collected (the primary end point), together with the type of mobilizing chemotherapy. The characteristics of the present series did not allow us to evaluate additional recognized variables like age and disease status (eg, chemotherapy sensitive or resistant disease) at the time of transplantation because only 16 patients (13.7%) were older than 40 years and most patients were responding. The negative effect of previous treatment load, although extensively evaluated in lymphoid malignancies, was addressed by few authors only in GCT.[20,21] The effect of the number of relapses (categorized 0-5) was only alluded to in a series of patients with solid tumors, which primarily focused on the effect of chemotherapy regimen and growth factor on mobilization.[20] According to our data, despite a global success in collecting CD34$^+$ cells in these patients, results obtained in the population of patients being treated later than second-line warrant confirmation in a larger sample size. A not negligible proportion of them actually failed either to mobilize

Figure 1 Box Plots Showing the Differences Across Regimens (Top) and Treatment Setting (Bottom, eg, First- and Second-Line vs. Third-Line or Beyond) Accounting for the 3 Mobilization Indexes Analyzed. Analysis Was Made by Considering the First Mobilization Attempt Only (Accounting for a Total of 116 Attempts-Patients)



Abbreviations: CTX = cyclophosphamide; PEI = cisplatin, etoposide, and ifosfamide; TPG = paclitaxel, cisplatin, and gemcitabine.

Table 2 Univariable Analyses of Type and Line of Chemotherapy Effect on Mobilization Indexes at First Attempt

	CD34+ Cell Peak Per µL		Time to CD34+ Cell Peak (Days)		Total CD34+ Cells Collected (× 10^6/kg), n[a]	
	Median	Interquartile Range	Median	Interquartile Range	Median	Interquartile Range
Induction Regimen						
CTX	99.0	62.0-164.5	13.0	12.0-14.0	10.6	9.2-15.0
PEI	95.0	59.5-179.2	16.0	15.0-19.0	14.8	12.3-17.3
TPG	67.0	51.5-102.5	10.0	9.0-11.0	10.0	8.2-14.3
Other	47.0	28.8-81.3	11.0	10.0-12.3	8.5	3.5-12.1
P Value at Anderson and Darling Test	.006		<.001		<.001	
Line of Treatment						
First- and Second-Line	94.0	56.0-169.0	14.0	12.0-16.0	12.2	9.4-16.2
≥Third-Line	35.0	21.5-70.0	11.0	9.5-15.5	8.1	2.2-12.2
P Value at Anderson and Darling Test	<.001		.003		.001	

Abbreviations: CTX = (7 g/m^2) cyclophosphamide; PEI = cisplatin, etoposide, ifosfamide; TPG = paclitaxel, cisplatin, gemcitabine.
[a]At first attempt.

Table 3 Multivariable Analyses Using Quantile Regression Models

	$CD34^+$ Cell Peak Per μL		Time to $CD34^+$ Cell Peak (Days)		$CD34^+$ Cells Collected ($\times 10^6$/kg), Total n[a]	
	Median Ratio	95% CI	Median Ratio	95% CI	Median Ratio	95% CI
Induction Regimen						
CTX vs. other	1.74	1.04-2.91	1.18	1.06-1.32	1.15	0.83-1.60
PEI vs. other	1.72	1.00-2.96	1.45	1.29-1.64	1.66	1.20-2.30
TPG vs. other	1.47	0.68-3.18	0.91	0.80-1.04	1.61	1.17-2.21
P value at likelihood ratio test	.052		<.001		<.001	
Line of Treatment						
First- or second-line vs. ≥third-line	1.68	0.98-2.88	1.00	0.90-1.12	1.63	1.08-2.44
P value at likelihood ratio test	.028		.999		.008	

Abbreviations: CTX = (7 g/m^2) cyclophosphamide; PEI = cisplatin, etoposide, ifosfamide; TPG = paclitaxel, cisplatin, gemcitabine.
[a]At first attempt.

(26.7%) or to collect the planned amount of $CD34^+$ cells (46.6%). Taken together, the present results might be in favor of a preemptive collection of cells in patients who are judged to be at high risk of treatment failure, for example, those who are classified in the high or very high risk category according to Beyer, more than 70% of whom are expected to require further salvage attempts. Moreover, the outcomes are in line with the hypothesis of a trial anticipating the administration of HD chemotherapy in an earlier treatment setting (namely the pure second-line setting) in which mobilization and harvest of hematopoietic stem cells might be easier.

We also provided guidance on the timing of stem cell harvest in a disease for which the scarce available information relies on small numbers. Of note, we observed a significant difference in the optimal timing to collect $CD34^+$ cells depending on the regimen. A delay was observed with the use of PEI (here referred to in the modified Italian version, PEI, median of 16 days to $CD34^+$ cells per μL peak) compared with paclitaxel and ifosfamide (11 days, included with other regimens in the model), which are among the most frequently used regimens with this aim. The results also showed a significant relationship between the chemotherapy regimen used and the amount of cells that can be collected, favoring PEI over other mixed regimens, including paclitaxel plus ifosfamide (T-I). The use of HD-CTX, although it was successful to mobilize, should not be recommended because it was part of an inactive sequence of a randomized Italian study compared with PEB.[10]

Conclusion

We suggested that the collection of adequate numbers of $CD34^+$ cells allowing a rapid and sustained engraftment might be impaired in highly pretreated GCT recipients, because they need large amounts of cells to undergo multiple transplantation procedures and because of the decline of the mobilization potential induced by previous chemotherapy, particularly beyond the second-line. Although caution is needed when interpreting results because most patients accomplished their therapeutic program, this information should be included in the decision algorithm of patients relapsing after first-line chemotherapy together with recognized prognostic variables. In agreement with the results of a recent case series,[22] plerixafor was endowed with substantial mobilizing activity in highly pretreated patients that justifies the extension of its indication beyond the hematologic malignancies as another working hypothesis. Based on the present findings, the authors (A.N., principal investigator) are promoting a new retrospective study on behalf of the solid tumors working party of the European Group for Blood and Marrow Transplantation. This will help to enhance the quality control of transplantations in GCT and to gather a validation cohort for our findings.

Clinical Practice Points

- Scarce information is available regarding the $CD34^+$ cell mobilization issue in GCT.
- The administration of multiple cycles of HD chemotherapy with $CD34^+$ cell rescue is one of the most widely used options in the salvage attempt for relapsing cases.
- The amount of previous chemotherapy regimens was associated with decreasing possibilities to collect a sufficient aliquot of $CD34^+$ cells in our series.
- In particular, patients being treated beyond the second-line setting experienced a significant reduction of the mobilization parameters and, ultimately, the number of total $CD34^+$ cells harvested.
- This information might be relevant when planning the overall salvage treatment strategy in patients in whom PEB treatment has failed, and might provide the rationale of a trial aimed at registering plerixafor for poor-mobilizing patients with GCT.

Disclosure

The authors have stated that they have no conflicts of interest.

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Supplemental Table 1 Univariable and Multivariable Cox Model Analyses of Overall Survival

	Univariable Models			Multivariable Models		
	HR	95% CI	*P*[a]	HR	95% CI	*P*[a]
Lines of Treatment, ≥3 vs. 1-2	2.06	0.90-4.72	.086	1.48	0.60-3.62	.390
Conditioning Regimen, CBDCA-VP16 vs. Other	2.73	1.21-6.12	.015	2.42	1.01-5.79	.047
Number of $CD34^+$ Cells Reinfused, 9.2 vs. 5.5[b]	0.77	0.42-1.41	.255	1.01	0.51-1.98	.871

Abbreviations: CBDCA = carboplatin; HR = hazard ratio; VP16 = etoposide.
[a]Two-sided Wald test *P* value.
[b]Modeled as continuous variable; 9.2 and 5.5, respectively, the third and first quartile of CD34 distribution.