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A single prior course of BCNU-cisplatin chemotherapy has a significant deleterious effect on mobilization kinetics of otherwise untreated patients

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Summary:

Extensive prior treatment with cytotoxic agents is associated with impaired mobilization of hematopoietic cells. To assess the effect of a single course of standard-dose chemotherapy (CT), we compared the results of filgrastim-induced mobilization among two sequential groups of grade III–IV malignant glioma patients included in a hematopoietic transplantation program. The first group (21 patients) had never been treated with CT until 2 days after surgery, when they received a course of 100 mg/m^2 BCNU (IV) and 100 mg intracarotid cisplatin for cytoreduction (not for mobilization). At 1 month after this CT, they were mobilized with 12 μg/kg filgrastim. The second group (22 patients) was mobilized with the same dose of filgrastim directly after the surgery, without having ever received any prior CT. The blood level of CD34+ cells was significantly lower in the CT-treated patients, both on the fourth day of filgrastim (15 *vs* 36 cells $\times 10^6$/l; $P=0.01$) and on the fifth (25 *vs* 58 cells $\times 10^6$/l; $P=0.003$), as it was the number of CD34+ cells collected per apheresis (1.3 *vs* 3.5 $\times 10^6$/l; $P<0.0005$). The toxic effect of a single course of BCNU-cisplatin CT led to significant impairment of the filgrastim-induced mobilization response.

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Cytokine-induced mobilization of hematopoietic stem cells (HSCs) to the peripheral blood (PB) is largely used for cell collection prior to transplantation. Nevertheless, the response to standard mobilization regimens is highly variable among patients. At least part of this variability is related to the long-term consequences of prior chemotherapy (CT).

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Most antineoplastic agents are harmful to hematopoietic progenitor cells (HPCs), the bone marrow microenvironment or both.[1] Particularly, nitrosoureas such as carmustine (BCNU) have been shown to bestow a persistent toxic effect on primitive HSCs.[2] Extensively pretreated patients, specially those who have received large cumulative doses of alkylating agents, have been shown to mobilize less progenitor cells than those who are not so heavily pretreated.[3–5] However, little is known about the actual effect of a single course of standard-dose CT on the HSC mobilization ability of a cancer patient.

High-grade malignant glioma is a rapidly progressive neoplastic disease of the central nervous system (CNS). Despite use of the best therapeutic approaches currently available, it is associated with a median survival of less than a year. A particular feature of this tumor is that its clinical aggressiveness is almost exclusively due to rapid local growth in such a sensitive territory. Metastatic dissemination out of the CNS is a rare phenomenon, and bone marrow can be assumed to be tumor-free in virtually any patient with high-grade glioma. Therefore, a population of CT-naive patients with this neoplasm represents an optimum model for investigating factors influencing HSC mobilization.

We compared HSC mobilization kinetics in a group of previously CT-untreated glioma patients with a historical control of patients with the same diagnosis and, in the same situation, similarly mobilized, but who had been treated with only one course of cisplatin-BCNU CT. The goal was to assess how a single course of standard-dose CT might change the response to a filgrastim-based mobilization regimen achieved by an otherwise presumably healthy bone marrow.

Patients and methods

This analysis was performed as part of a therapeutic trial of HPC transplantation for patients with grade III and IV malignant glioma achieving optimal cytoreduction by surgery. With the first design of the program, 2 days after the surgery, the patient received a course of 100 mg/m^2 carmustine (BCNU) intravenously plus 50 mg/m^2 cisplatin by the intracarotid route. It should be noted that the purpose of this CT was cytoreduction, not mobilization. At

1 month after this CT, patients were mobilized with filgrastim, 12 μg/kg subcutaneously, and underwent PB HPC collection by apheresis. In a further review of the program, the course of standard-dose BCNU and cisplatin mentioned was eliminated, and patients commenced mobilization directly 2 weeks after the surgery, with the same dose of filgrastim (see Figure 1). The goal of this protocol amendment was two-fold: to reduce the delay for the beginning of the planned post transplant radiation therapy and to attempt to improve the HSC mobilization results, found to be surprisingly poor in an interim analysis. We compared the mobilization, collection and engraftment kinetics achieved in the patients entered into this program before and after this change.

HPC collection

Daily leukaphereses were performed from the fourth day of filgrastim administration. A dual-lumen central catheter was placed specifically for this purpose. The collections were performed with either a Cobe Spectra (software version 4.0, MNC collection program) or a Fenwall CS3000plus (5-special program, with reverse of flow during spillover and 50-ml chamber) cell processor. Between 2.5 and 5 blood volumes were processed per session. A minimum of two leukaphereses was scheduled. All products collected were cryopreserved, and then thawed and infused during transplantation, with no back-up product retained.

Conditioning regimen and further management

The conditioning CT consisted of 900 mg/m^2 BCNU intravenously, plus 50 mg/m^2 cisplatin by the intracarotid route. HPCs were re-infused 48–72 h after the end of the high-dose CT. Radiotherapy to both tumor cavity and the margins was administered from the seventh day post infusion. After reaching a dose of 55 Gy, the tumor was exposed to a further 20–25 Gy of stereotaxic radiosurgery.

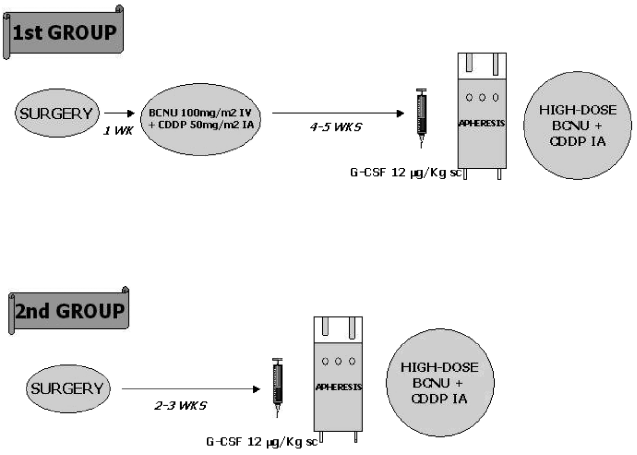

Figure 1 Therapeutic schedule in the two groups of patients compared. With the first design of program, the patients received a course of cytoreductive CT after surgery, and were mobilized with filgrastim after they had recovered from it. After protocol revision, the course of CT mentioned was eliminated and patients commenced mobilization directly after surgery. WK/WKS: week/weeks; BCNU: carmustine; CDDP: cisplatin; IV: intravenous route; IA: intra-arterial route.

Prophylactic single-donor platelet transfusions were administered when the PB count fell below 20×10^9/l. Red blood cell transfusions were given if the hemoglobin level was lower than 8.5 g/l.

Statistical analysis

As the distribution of most variables was significantly different from normal (tested with Kolmogorov–Smirnoff's test with Liliefor's Correction), descriptive statistics were given as median with the interquartile range (IQR) in brackets, and Mann–Whitney's test was used for group comparisons. A SPSS 9.0 statistical package was used for data management and statistical calculations.

Results

A total of 46 consecutive patients entered this program from July 1997 to September 1999. Three of them were excluded from the analysis because of different violations of the mobilization program. Among the remaining 43 patients, the first 21 received the course of standard-dose BCNU plus cisplatin 1 month before mobilization, and the remaining 22 were mobilized directly after surgery. The main characteristics of the patients in both groups are outlined in Table 1.

The median levels of CD34+ cells reached in PB were significantly lower in the group previously treated with one course of CT, both after 4 days of filgrastim administration (15 *vs* 36×10^6/l cells; $P = 0.01$) and after 5 days (25 *vs* 58×10^6/l cells; $P = 0.003$). The median peak PB CD34+ cell count was 27×10^6/l cells (IQR: 33) in the treated group *vs* 57×10^6/l cells (IQR: 42) among the untreated ($P = 0.003$) (Figure 2).

Cell-collection results were similarly different between the two groups. The median absolute number of CD34+ cells collected was 3.4×10^6/kg (IQR: 4) in the group previously treated with CT *vs* 6.9×10^6/kg (IQR: 8.2) in the untreated group ($P < 0.0005$). The CD34+ cell yield per processed blood volume was 0.5×10^6/kg (IQR: 1) among the treated *vs* 1.3×10^6/kg (IQR: 1.7) in the untreated patients ($P < 0.0005$). Table 2 summarizes the collection results achieved.

Despite the small number of patients in each group, these differences remained significant or nearly significant after

Table 1 Patient characteristics

	Previous CT	*No previous CT*	*Overall*
Number of patients	21	22	43
Age (median)	45	42	44
Karnofsky (median)	70%	80%	70%
Type of glioma			
Glioblastoma multiforme	14	12	26
Anaplastic astrocytoma	4	8	12
Anaplastic oligodendroglioma	2	1	3
Anaplastic oligoastrocytoma	0	1	1
Gliosarcoma	1	0	1

CT: chemotherapy.

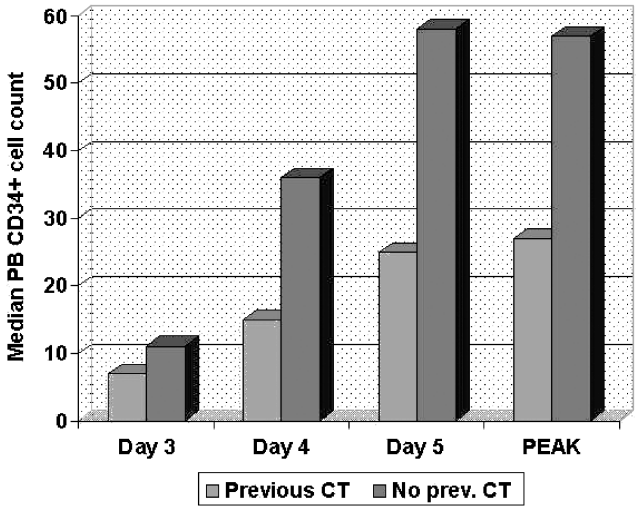

Figure 2 Median level of PB CD34+ cells ($\times 10^6$/l) reached along the mobilization treatment. CT: chemotherapy; PB: peripheral blood.

Table 2 Overview of collection results

	Previous CT	*No previous CT*	*P*
Total CD34+ cells collected[a]	3.4	6.9	<0.0005
CD34+ cells collected the fifth day of mobilization[a]	1.9	4.4	0.001
CD34+ cell yield per apheresis[a]	1.3	3.4	<0.0005
CD34+ cell yield per blood volume processed[a]	0.5	1.3	<0.0005
Number of aphereses needed	3	2	<0.0005

[a] $\times 10^6$/kg; CT: chemotherapy.

adjusting the analyses for the pathological stage of the tumor. The median peak PB CD34+ cell counts were 29 *vs* 50×10^6/l cells ($P = 0.05$) among stage IV (glioblastoma multiforme) patients and 24 *vs* 64×10^6/l cells ($P = 0.017$) among stage III (anaplastic astrocytoma or oligodendroglioma). The median absolute numbers of CD34+ cells collected were 3.7 *vs* 6.3×10^6/kg ($P = 0.026$) in the stage IV patients and 2 *vs* 7.8×10^6/kg ($P = 0.007$) among stage III patients. The CD34+ cell yields per processed blood volume were 0.5 *vs* 1.2×10^6/kg ($P = 0.006$) among stage IV, and 0.6 *vs* 1.6×10^6/kg ($P = 0.014$) in stage III patients.

Conversely, the engraftment kinetics were similar in the patients previously treated with CT as compared to those never treated before the transplant. The median numbers of days with less than 0.5×10^9/l granulocytes were 5 *vs* 5 ($P = 0.89$) and median numbers of days post infusion to the last platelet transfusion were 9 *vs* 9 ($P = 0.94$). Median numbers of platelet transfusions needed were 1 *vs* 1 ($P = 0.37$), and median number of red blood cell packs transfused were 2 *vs* 2 ($P = 0.35$).

Discussion

The small likelihood of bone marrow tumor infiltration in untreated stage III–IV malignant glioma makes this population of patients especially suitable for a study of factors influencing mobilization kinetics. In this setting, the behavior of patients never treated with CT from our series should be superimposable upon that of healthy individuals. In fact, the mobilization and collection results achieved in this group are similar to those described after filgrastim mobilization of normal donors for allogeneic PB HPC transplantation.[6,7]

Exposure to cytotoxic agents has been said to produce different degrees of residual marrow damage.[2] Neben *et al*,[8] in a model of murine syngeneic transplantation, demonstrated a decrease in HSC self-renewal in mice previously exposed to drugs such as BCNU or cisplatin. In the clinical setting, Drake *et al*[9] described a scoring system to grade the amount of previously received CT in patients eligible for HPC transplantation. Previous exposure to drugs classified as 'factor 4' in their score (the most toxic for stem cells, including BCNU) emerged as the only significant factor influencing CD34+ cell yield in a multivariate analysis. Likewise, Gandhi *et al*[10] and Clark *et al*[11] found similar results in two independent series of patients using Drake's Score. Other studies analyzing the influence of different variables on mobilization and collection also found worse results among heavily pretreated patients,[3–5] with a remarkable effect among patients treated with regimens including BCNU in combination with melphalan such as dexa-BEAM.[11] Nevertheless, all these studies analyzed patients treated with several courses of combination CT and, therefore, drew conclusions mainly associated with this very fact, such as the significant negative effect of more than six previous courses of CT, more than 24 months of prior therapy or a score of previous CT greater than 60.[3–5] To the best of our knowledge, no data are available about the actual effect of a single course of CT administered to untreated patients with an otherwise healthy bone marrow. The median summation score of previous CT in the study by Drake *et al*[9] was 38, while that of the patients treated with the single BCNU-cisplatin course in our series was only 6.

Based on experimental data and theoretical considerations, the nitrosoureas have been classically considered more toxic for the stem cell than cisplatin.[9–12] Nonetheless, the former drug is far from being harmless to the bone marrow.[8,13] Lee *et al*[13] found prior treatment with cisplatin to be a significant independent factor predicting worse HPC collection. Cisplatin was administered by the direct intra-arterial route in our study, but this does not rule out the possibility of a systemic effect of this drug contributing to the hematopoietic toxicity of BCNU.

The hematopoietic depression induced by the nitrosoureas is characteristically delayed.[14,15] In the clinical setting, it is not unusual to need an interval of 5–6 weeks between BCNU-based CT courses. This characteristic could provide a possible explanation for the dramatic impairment of mobilization capacity observed in our study among the treated patients. The generally accepted interval of 1 month from the last CT course to the beginning of mobilization was not enough after nitrosourea-based CT.

Several studies have described the effect of both HPC dose and prior CT received on engraftment

kinetics.[3,5,12,16,17] Early leukocyte and platelet recovery is reasonably guaranteed in most patients receiving more than 2×10^6/kg CD34+ cells, providing they had not been extensively pretreated.[3,10] Even though the previously treated patients in our series received a significantly lower dose of CD34+ cells, the median dose in this group was 3.4×10^6/kg and it was above 2×10^6/kg in 16 cases of 21 (76%). This number of HPC seems to have been enough for early hematopoietic recovery, and, thus, no difference in engraftment kinetics was found with respect to the previously untreated group.

In conclusion, even a single course of standard-dose CT, containing stem cell toxic agents, had a significant negative effect on filgrastim-based HPC mobilization. As a direct consequence, there was a significantly greater difficulty in collecting stem cells, although it did not preclude the procurement of an appropriate amount in most cases. This effect seems to be the consequence of a certain degree of bone marrow damage induced by the small cumulative amount of CT. This toxicity was sufficient to reduce the mobilization response, but not to preclude early engraftment, provided that an appropriate cell dose was given at transplantation.

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