Haematologica 2000; 85:160-166
original paper

Malignant Lymphomas

VACOP-B, high-dose cyclophosphamide and high-dose therapy with peripheral blood progenitor cell rescue for aggressive non-Hodgkin's lymphoma with bone marrow involvement: a study by the non-Hodgkin's Lymphoma Co-operative Study Group

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ABSTRACT

Background and Objectives. **Sequential treatment with the addition of high-dose therapy (HDT) and peripheral blood progenitor cell (PBPC) rescue has been reported to be active as front-line therapy in aggressive non-Hodgkin's lymphoma (NHL) with bone marrow (BM) involvement. We designed an intensive sequential therapy as front-line therapy in this subset of patients and conducted a phase II study.**

Design and Methods. **Patients with aggressive non-Hodgkin's lymphoma and BM involvement at diagnosis received 8 weeks of VACOP-B chemotherapy as induction therapy. The second phase included high-dose cyclophosphamide (HDCY) (7 g/m²) with granulocyte colony-stimulating factor (G-CSF) followed by leukaphereses. The third phase included HDT according to the BEAM protocol or melphalan (140 mg/m²) plus total body irradiation (8 Gy in a single dose).**

Results. **Forty patients were included in the study. According to the *intention-to-treat*, after VACOP-B, 11 (27.5%) and 22 (55%) patients achieved complete remission (CR) and partial remission (PR), respectively. Thirty-four received HDCY. After HDCY, 18 patients (45%) were in CR and 13 (32.5%) in PR. Twenty-nine underwent HDT plus peripheral blood cell rescue (PBPC) rescue. At the completion of treatment 29 patients (72.5%) were in CR, and 3 patients (7.5%) in PR. The actuarial 3-year overall survival, disease free survival and failure free survival are 48%, 55% and 40%, respectively. Overall severe toxicity was 7.5%.**

Interpretation and Conclusions. **This phase II study suggests that the intensified treatment described is feasible and active in aggressive NHL with BM involvement. A randomized trial is now underway to test this approach.**

Key words: high-dose therapy, aggressive non-Hodgkin's lymphoma, bone marrow involvement.

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Non-Hodgkin's lymphomas (NHL) are a heterogeneous group of tumors which vary in clinical behavior and response to treatment.[1] The histology of bone marrow (BM) involvement at diagnosis may have prognostic clinical implications. About 20-25% of patients with aggressive NHL have initial BM involvement which predicts a poor response rate to conventional treatment and a reduced survival.[2-6] In these patients, a complete remission (CR) rate of about 30%-50% is expected with a 3-year probability of survival ranging from about 20% to 40%.[2-6] A recent study reports a 3-year survival and failure-free survival (FFS) of 36% and 23%, respectively,[6] decreasing at 5 years to 30% and 12%.

Many prognostic risk-factor models have been proposed for aggressive NHL but results of multiparametric analyses, including the International Prognostic Index, do not single out BM involvement from other extranodal sites.[7-9] The International Prognostic Index has confirmed that BM involvement is no more important than other extranodal sites, but definitively shares the same prognostic significance predicting a poor outcome.[9] However, many factors can characterize the prognostic significance of BM involvement such as cytology, pattern of infiltration, and extent of involvement.[2-6] These variables and their integration in the International Prognostic

Index (IPI) could identify groups of patients with a poor probability of survival and FFS.[6]

High-dose therapy (HDT) with autologous stem-cell rescue has been demonstrated to be effective and feasible in refractory or relapsed NHL,[10,11] even in patients with persistent BM involvement.[12,13] Recently, sequential therapy with HDT and peripheral blood progenitor cell (PBPC) rescue as front-line therapy has been reported as being highly effective in aggressive NHL with BM involvement giving a long-term probability of survival and FFS of 53% and 45%, respectively.[14]

In 1991, the *Italian Non-Hodgkin's Lymphoma Co-operative Study Group* (NHLCSG) began a multicenter phase II study on patients with aggressive NHL with BM involvement at diagnosis. Patients received etoposide, doxorubicin, cyclophosphamide, vincristine, prednisone, and bleomycin (VACOP-B),[15] followed by high-dose cyclophosphamide (HDCY) and HDT with PBPC rescue.

The principal aim of this study was to clarify the role of an intensified sequential front-line therapy. We also planned to explore, in terms of outcome, the relationship between the type of bone marrow involvement and other well established adverse prognostic factors, including age-adjusted IPI.

Design and Methods

Eligibility criteria

This study was a prospective co-operative phase II study with 9 participating centers from the NHLCSG. The study began in October 1991 and enrolment finished in December 1994. Study eligibility criteria were as follows: patients aged 15-60 years, with diffuse intermediate or high-grade non-Hodgkin's lymphomas according to the Working Formulation (WF)[16] (excluding lymphoblastic lymphoma and Burkitt's lymphoma), BM involvement at diagnosis, normal renal, pulmonary, cardiac, and hepatic function unless abnormal because of disease involvement, and written informed consent. Pre-treated patients or those with a positive serology to human immunodeficiency or hepatitis B or C virus were considered ineligible.

Staging procedure

Staging included routine blood chemistry tests, blood cell counts and differentials, ECG, and chest X-ray. Extent of disease was confirmed by physical examination, bilateral iliac crest bone marrow biopsies and computed tomography of the chest, abdomen and pelvis. Magnetic resonance imaging and radionuclide scans were performed when required. A lumbar puncture with CSF examination was performed in all cases. The number of extra-nodal sites and the diameter of the largest tumor mass were determined.

Treatment

Treatment was in three phases:

1) the induction phase consisted of 8 courses of VACOP-B chemotherapy according to the original treatment scheme.[15] Patients in CR, or in partial remission (PR), without improvement of response observed during the last two courses of chemotherapy, received the second phase of treatment. Patients in PR with improvement of response received 4 additional weeks of VACOP-B. Non-responders (NR) or patients with progression of disease (PD) underwent phase II therapy after the first month of treatment.

2) The second intensified phase included cyclophosphamide at the total dosage of 7 g/m^2 in 5 divided doses (Table 1). From day +1 after HDCY patients received continuous infusion of G-CSF at the dosage of 5 µg/kg.

3) The third phase included HDT. Patients received a chemotherapy over 6 days according to the BEAM protocol,[10] or melphalan (140 mg/m^2 in a single dose) on day -3 and total body irradiation (8 Gy in a single dose) on day -1. Peripheral blood progenitor cells (PBPC) were reinfused on the day after HDT, nominally day 0 of the transplant procedure. Patients did not receive growth factors after PBPC rescue.

On completion of treatment, patients with residual masses received involved field radiotherapy.

Mobilization, peripheral blood progenitor cell collection and high-dose therapy

In all cases, a central venous catheter was inserted after the final course of VACOP-B. From day +1 after HDCY, patients received a continuous infusion of G-CSF at the dosage of 5 µg/kg. Leukaphereses began when white blood cells increased to > 1.0×10^9/L to collect more than 5×10^6 CD34$^+$ cells per kilogram of body weight. Growth factors were stopped after the last leukapheresis. In cases in which repeated leukaphereses were required the administration of growth factors was discontinued once the leukocyte count reached 30×10^9/L. Hematopoietic precursors (CD34$^+$ cells and CFU-GM) were evaluated as previously reported.[17] The apheresis product was cryopreserved as described elsewhere.[12]

Table 1. Treatment protocol and time schedule for HDCY (7 g/m^2).

Hour	*Cyclophosphamide (CY)*	*Uromitexan*
0	CY 1.4 g/m^2/i.v./1 hour	
1		1.5 g/i.v./bolus
3	CY 1.4 g/m^2/i.v./1 hour	
4		1.5 g/i.v./bolus
6	CY 1.4 g/m^2/i.v./1 hour	
7		1.5 g/i.v./bolus
9	CY 1.4 g/m^2/i.v./1 hour	
10		1.5 g/i.v./bolus
12	CY 1.4 g/m^2/i.v./1 hour	
13		1.5 g/i.v./bolus
15/18/21/24/ 27/30/33		1.0 g/i.v./bolus

Hyper-hydration: solution III + KCl 30 mEq/L + $NaHCO_3$ 30 mEq/L (3,000 mL/m^2/24 h), furosemide 20 mg before first CY i.v. administration. Urine pH >7, assessment 2 hourly, $NaHCO_3$ 5% as necessary. Diuresis every 2 hours, 12-hourly balance to be corrected with furosemide as required. Acetazolamide 250 mg p.o. every 6 hours, starting 4 hours before CY. Ondansetrone 8 mg before CY and every 8 hours i.v. as required. Start of hyper-hydration at least 12 hours before CY.

Table 2. Characteristics of 40 patients.

Characteristics	*No.*	*%*
Age, years		
Median	48	
Range	19-56	
Sex		
Male	25	62.5
Female	15	37.5
Performance status		
0	25	62.5
1	10	25.0
≥2	5	12.5
Histology		
Diffuse mixed (F)	13	32.5
Diffuse large-cell (G)	15	37.5
Large-cell immunoblastic (H)	6	15.0
Other		
Anaplastic/Ki-1 (K)	6	15.0
Immunophenotype		
B	33	82.5
T	6	15.0
Null	1	2.5
BM involvement	40	100.0
Symptoms		
A	20	50.0
B	20	50.0
Spleen involvement	10	25.0
Extranodal sites		
1	12	30.0
≥2	28	70.0
Bulky disease ≥10 cm		
No	33	82.5
Yes	7	17.5
Lactate dehydrogenase		
NV	20	50.0
>1 NV	20	50.0
International Index		
Low-intermediate	20	50.0
Intermediate-high	14	35.0
High	6	15.0

Abbreviations: NV, normal value

Following HDCY and during phase 3 including HDT with PBPC autografting, patients were treated in single ward rooms without laminar air flow. Patients showing clinical evidence of herpes simplex were treated with acyclovir. Supportive care procedure as described in detail elsewhere[10] was adopted during autografting. Platelet transfusions from individual donors were given for platelet counts under 20×10^9/L. Leukocyte-free erythrocyte concentrates were administered when the hemoglobin level fell below 8 g/dL. All blood products were irradiated (20 Gy).

Assessment of response

All patients underwent re-staging after VACOP-B chemotherapy, and after HDCY. Following HDT, patients were re-staged every three months during the first year, every six months in the second year, and annually thereafter. A complete remission (CR) was defined as the complete disappearance of the disease. On completion of treatment patients with a residual radiologic mass and no signs or symptoms of active disease, who maintained a stable condition for at least 3 months, were judged to have a CR. A partial remission (PR) was defined as a more than 50% reduction, and non-response (NR) a less than 50% reduction in tumor mass and in BM infiltrate. Patients who received consolidation radiotherapy were assessed for response on completion of therapy. The toxicity of conventional chemotherapy and HDT was evaluated according to World Health Organization (WHO) criteria.

Statistical methods

A sample of 40 patients[18] was considered sufficient to provide this trial with an adequate power (i.e. 80%) to detect a difference of at least 20% in the CR rate in comparison with previously reported data.[6]

Patients were registered by telephone at the Trial Office of the National Cancer Institute in Genoa, Italy.

Analysis was based on status of disease on December 30th, 1998. According to intention-to-treat, we analyzed the results of all patients who entered the study.

Survival was measured from the date of registration to the date of death or last follow-up evaluation. Failure-free survival (FFS) was calculated from the date of registration to the date of relapse, progression, death or the last follow-up evaluation. Disease-free survival only applied to patients who achieved a complete remission. Duration was calculated from the time of CR assessment to the date of relapse, death or last follow-up evaluation confirming the patient to be free of disease.

Actuarial curves were estimated according to Kaplan and Meier's method and compared by the log-rank test.[19]

Survival analysis according to a number of prognostic factors was performed: performance status, symptoms (A vs. B), bulky disease (≥ 10 cm vs. others), number of extranodal sites (1 vs. ≥ 2), lactate dehydrogenase (LDH) level (≤ 1× vs. > 1× normal value).

BM biopsies obtained at the time of diagnosis were reviewed and assessed for univariate analysis as follows: extent of BM involvement, less than 30%, and less than 50% vs the others; percentage of large cells in the infiltrate, less than 10%, and less than 30% vs the others; pattern of lymphoma involvement, diffuse plus interstitial vs the others.

Survival, DFS and FFS were also retrospectively analyzed according to the *International Non-Hodgkin's Lymphoma Prognostic Factors Project* (International Index). The results were adjusted for age < 60 years. Patients were subdivided into four groups (low-, low-intermediate, intermediate-high, high-risk) according to the presence of zero, one, two or three risk factors, respectively. These risk factors were: performance status > 1, LDH level > 1 × normal value, and stage > II .

Results

Patients' characteristics

Forty successive patients entered the study and their pre-treatment characteristics are reported in Table 2. The patients' median age was 48 years (range, 19 to

Table 3. Extent and cytology of BM involvement by tumor at diagnosis in 39 patients with aggressive NHL.

		BM involvement (%)					*Large cells (%)*				
Histology		*<10*	*10-29*	*30-49*	*50-69*	*≤ 70*	*<10*	*10-29*	*30-49*	*50-69*	*≥ 70*
Diffuse mixed	13	0	6	4	2	1	10	3	0	0	0
Diffuse large-cell	15	2	8	2	2	1	9	4	1	1	0
Large-cell immunoblastic	5	0	3	0	0	2	3	0	0	0	2
Anaplastic Ki-1	6	0	1	0	4	1	2	0	0	3	1
Total (%)	39	2 (5)	18 (46)	6 (15)	8 (21)	5 (13)	24 (61)	7 (18)	1 (3)	4 (10)	3 (8)

56). The histology was diffuse mixed (F/WF) in 13 patients, diffuse large-cell (G/WF) in 15, large-cell immunoblastic (H/WF) in 6, and anaplastic Ki-1 (K/WF) in 6 patients. Fifty percent of patients had B symptoms, 70% of these had two or more extranodal sites, while 50% had an above normal level of lactate dehydrogenase. According to the International Index 50% of patients were classified as low-intermediate risk, 35% as intermediate-high risk and 15% as having high-risk.

Cytology and extent of BM involvement by lymphoma was determined semi-quantitatively in patients showing a median BM involvement of 27.5% (range 8% to 100%). BM biopsies at diagnosis from 39 out of 40 patients were available for complete analysis (Table 3). Two patients (5%) showed a BM infiltrate of less than 10%, 18 (46%) from 10% to 29%, 6 (15%) from 30% to 49%, 8 (21%) from 50% to 69%, and 5 (13%) more than 70%. This distribution indicates that the majority of patients, 26 (66%), had less than 50% of their BM replaced by lymphoma.

The majority of patients, 24 out of 39 (61%), had a BM infiltrate containing less than 10% of large cells, with predominant small-cell infiltration. Fewer than 10% of large cells were found in 10 out of 13 patients (77%) with diffuse mixed (F/WF), in 9 out of 15 (60%) with diffuse large-cell (G/WF), in 3 out of 5 (60%) with large cell immunoblastic (H/WF), and in 2 out of 6 (33%) with anaplastic/Ki-1 (K/WF) NHL. Large cells were not seen in 5 cases of diffuse mixed and in one case of diffuse large-cell NHL. Eight patients showed only large-cells in their BM (G=3, H=2, K=3).

The pattern of infiltration was nodular in 9 (23%) patients, paratrabecular in 8 (20.5%), diffuse in 8 (20.5%), interstitial in 4 (10%), and mixed in 10 (26%). A nodular pattern was seen in all categories of patients (F=31%, G=20%, H=17%, K=17%). A paratrabecular pattern was only present in groups F/WF (15%) and G/WF (40%). The diffuse pattern was more frequent in the large-cell NHL categories than in the mixed-cell type (F=8%, G=7%, H=50%, K=50%). Ten patients (26%) had a mixed infiltrate: diffuse-nodular 4 patients, interstitial-nodular 3 patients, diffuse-paratrabecular 1 patient, nodular-paratrabecular 2 patients. The diffuse pattern of infiltrate correlated with a more extensive involvement and a higher percentage of large cells in BM.

Response and toxicity

Forty patients were given induction-phase treatment and received a median of 8 courses of VACOP-B (range 4 to 12). Following therapy, 11 (27.5%) and 22 (55%) patients achieved CR and PR, respectively. Seven patients (17.5%) progressed (4 patients) or did not respond (3 patients) to treatment. The following grade 3 and 4 WHO toxicities were observed: leukopenia and granulocytopenia (10% of patients), anemia (7.5%), and infection (7.5%). Twenty per cent of patients suffered grade 1 or 2 mucositis.

Six patients were withdrawn from the subsequent intensification phase because of progressive disease (4 patients) or refusal while in first CR (2 patients). These last two patients are alive and well, 62 and 63 months after VACOP-B.

At a median time of 24 days (SE: ±10.4) from the last course of VACOP-B, 34 patients received HDCY plus growth factors. Thirty-one patients underwent leukaphereses on a median of day 12 (SE: ±0.58). Three patients did not continue the procedure: two because of progression and the third because of HDCY-related cardiac death while in PR. A median of 3 aphereses (SE: ±0.42) was performed. The median number of mononuclear cells harvested was $6.1x10^8$/kg (SE: ± 1.78), the median number of $CD34^+$ cells harvested was $16.3x10^6$/kg (SE: ± 8.2), and the median number of CFU-GM was $36.7x10^4$/kg (SE: ± 41.4). Following HDCY, another 7 patients with PR went on to achieve CR. Therefore, at the completion of treatment, 18 patients (45%) achieved CR, 13 (32.5%) PR, 8 were NR or PD, and one had died of extrahematologic toxicity. All patients suffered hematologic HDCY-related toxicity with pancytopenia (leukocyte nadir $0.1\text{-}0.2x10^9$/L). Twenty-one out of 34 patients (62%) required erythrocyte and/or platelet concentrate transfusions before or during leukaphereses. All patients experienced nausea and vomiting (grades 1- 3). Five patients (15%) presented fever of unknown origin. Two patients (6%) showed transitory grade 2 liver toxicity. Among patients achieving a CR, one (3%) experienced grade 3 lung toxicity with pulmonary fibrosis, and another grade 4 liver and kidney toxicity. This last patient went on to develop progressive disease and later died. These 2 patients were excluded from HDT. One partial responder died of treatment-related cardiac failure. In conclusion, 5 patients did not receive HDT. This was due to toxicity (2 CR patients); PD (2 patients); and death (1 patient).

Twenty-nine patients received HDT with PBPC rescue at a median time of 60 days from HDCY (SE: ± 9.0). Eleven out of 40 patients (27.5%) did not undergo the BEAM regimen (received by 15 patients)

or melphalan/TBI (received by 14 patients) with PBPC rescue for the various reasons reported above.

Following HDT and PBPC rescue, 11 additional patients (27.5%) achieved CR (10 PR patients and 1 PD patient). Therefore, at the completion of treatment, 29 patients (72.5%) were in CR, 3 patients (7.5%) were in PR, and the remaining patients had progressive disease, no response or were dead. Three patients received involved field radiotherapy to residual masses.

All transplanted patients had pancytopenia. Marrow engraftment occurred in all. The median time necessary to establish a self-sustaining granulocyte recovery greater than 0.5×10^9/L was 10 days (SE: ±0.82), while the median time for platelet recovery > 20×10^9/L was 11 days (SE: ±1.4). All patients experienced nausea and vomiting (grade 2 and 3). Mucositis was observed in all patients. During the aplastic phase, infection requiring antibiotic therapy occurred in 10 patients: (grade 3, 4 patients; grade 2, 3 patients; grade 1, 3 patients). Three patients developed grade 1 or 2 liver toxicity. No treatment-related mortality was observed. One patient died of myocardial infarction 2.5 years after the completion of therapy.

According to *intention-to-treat*, the overall response rate was 80% (CR=72.5%, PR=7.5%).

Survival analysis

Twenty-two patients (55%) died of early or late progressive disease, two patients (1 in CR, 1 in PR) died of cardiac failure, one, while in CR, two years after HDT, one after HDCY. Up to now 16 (40%) out of 40 patients have survived with a median observation time of 60 months (range 43-79 months). Sixteen (55%) out of 29 patients in CR relapsed in a median time of 8 months (range 3-43 months). At present 13 patients are disease-free after a median observation time of 53 months (range 37-69 months).

The actuarial overall 3-year survival is 48%. The estimated 3-year DFS is 55%. The 3-year FFS is 40%. The projected curves show survival, DFS and FFS at 5 years of 42% (95% confidence limit, 36% to 47%), 39% (95% confidence limit, 32% to 45%) and 34% (95% confidence limit, 29% to 39%), respectively (Figure 1).

Prognostic factors for survival, DFS and PFS were studied using univariate analyses. Prognostic factors analyzed at diagnosis were as follows: performance status, B symptoms, bulky disease, number of extranodal sites, LDH elevation. Only B symptoms proved to be significant in terms of survival (p=0.04) and patients without B symptoms had the best outcome. According to univariate analyses of DFS and FFS, no negative prognostic factors predicting a poor outcome were found.

Extensive univariate analysis was performed to correlate extent, cytology and pattern of BM involvement and survival, DFS and FFS. This analysis did not demonstrate any statistical correlation or trend.

Stratification of patients according to IPI did not show any significant difference in terms of survival and of FFS.

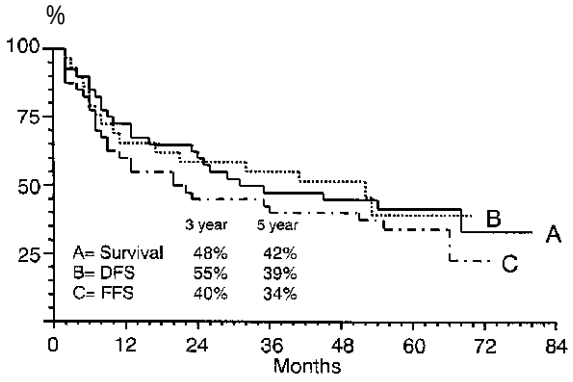

Figure 1. Overall survival (A), disease-free survival (B) and failure-free survival (C) of patients who underwent treatment.

Discussion

Despite the controversy concerning the statistical significance of bone marrow involvement at diagnosis in aggressive NHL,[7-9] several observations have reported a poor prognosis in this category of patients with a lower CR rate and high relapse rate.[2-6] Prognostic factors and bone marrow involvement, as defined by cytology, pattern and extent of infiltrate, seem to reduce the probability of cure. Therefore the main objective of this study was to test the feasibility and effectiveness of an intensified sequential therapy and subsequently analyze the relationship between negative factors, BM status and outcome. As reported above, with the use of conventional chemotherapy, a CR rate of about 30%-50% was expected.[2-6] However, relapse was common and 3- and 5-year probabilities of survival were 36% and 30%, respectively.[6] The same authors reported a 3- and 5-year FFS of 23% and 12%, respectively.

Recently, the use of a new strategy of high-dose sequential therapy in patients with BM involvement at diagnosis was reported to improve the CR rate and survival.[14] Our sequential therapy, including a short standard therapy followed by two intensified phases, achieved CR in 72.5% of patients. Of these, 27.5% were observed after 8 courses of VACOP-B, 17.5% after HDCY, and 27.5% after HDT and PBPC rescue. The low CR rate observed with the VACOP-B regimen could be linked to the poor prognosis of these patients.[5,6] According to our results, 17 out of 22 patients (77%) who were in PR after VACOP-B achieved CR using two intensified therapy phases. Three patients received involved field radiotherapy at completion of treatment. Two of these had progression and one was judged to be in CR with a treatment-related residual fibrotic mass. Projected actuarial curves show a 3-year probability of survival, DFS, and FFS of 48%, 55% and 40%, respectively. At 5 years the probability is 42%, 39% and 34%, respectively. These results, similar to those reported by Vitolo *et al.*,[14] suggest that sequential HDT may improve outcome in this subset of patients and needs to be evaluated in a prospective phase III trial.

In spite of our encouraging results, the problem of patients in PR after front-line treatment remains unresolved. The use of conventional treatment in these patients is disappointing with an expected CR

rate of about 20-30% and a long-term survival rate of less than 10%.[5,20-22] Recently, patients in PR after three courses of CHOP were randomized to receive five additional CHOP courses or HDT and autologous bone marrow transplantation.[23] This study did not show any advantage in terms of CR rate, survival, DFS or event-free survival in favor of patients treated with HDT and ABMT. More recently, patients in a multicenter study with an early PR after MACOP-B or F-MACHOP were randomized to receive conventional treatment or HDT and autologous bone marrow transplantation24. This last study showed a statistical improvement in terms of overall response (CR + stable PR) and a positive trend in terms of survival in favor of high-dose treatment with a response rate of 96% versus 59% ($p < 0.001$), five-year survival (73% versus 59%) and PFS (73% versus 52%).

In our study, sixteen out of 29 (55%) CR patients relapsed in a median time of 10.5 months (median observation time 53 months). Although the high CR rate seems to confirm that high-dose sequential therapy is able to improve outcome for this category of patients, the high rate of relapse suggests that additional and new treatments are badly needed. An important point for discussion is related to the possible contamination of aphereses by tumor cells, which could partially explain the high relapse-rate. A relationship between status of bone marrow (positive or negative for tumor cells), status of PBPC, and probability of maintaining CR after HDT plus PBPC rescue has been previously discussed in similar categories of patients. Survival was significantly better in patients who received tumor-negative harvests and worse in patients who received contaminated harvests.[25] Unfortunately we did not perform a molecular study in our patients in order to detect tumor cells in the harvests. However, according to data reported elsewhere, a relatively good outcome can be achieved with HDT and PBPC rescue, even in patients with a significant marrow tumor burden.[12,13,25]

The effectiveness of CY in patients with NHL is well established.[26,27] We, therefore, explored the usefulness of HDCY at 7 g/m^2. At this dosage, CY doubles the number of CFU-GM without the administration of haematopoietic growth factors.[28] The association of growth factors and HDCY accelerates hematopoietic recovery and significantly increases the yield of CFU-GM[29] and of CD34$^+$ cells[30] which can be used for autografting. In the present study, the use of CY at the dosage of 7 g/m^2 plus growth factors permitted an effective PBPC collection with a rapid and sustained engraftment in all patients. It also demonstrated that HDCY is a highly effective drug in reducing tumor burden with 7 out of 22 (32%) patients in PR achieving CR.

One of the major issues concerning HDCY treatment is procedure-related morbidity and mortality. In our study, one patient died of cardiac failure and two patients had to be withdrawn from treatment after HDCY because of extra-hematologic toxicity. However, the overall severe toxicity of 7.5% observed in our study is comparable to that observed with a third-generation chemotherapy regimen[5] or ABMT procedure.[10]

This study confirms the large difference between BM and lymph node histology seen in previous reports.[2-4,6] The majority of patients (66%) had less than 50% of BM replaced by lymphoma, and 61% of patients had less than 10% of large cells in the BM with predominant small-cell infiltration. A small number of large cells is more frequent (77%) in diffuse mixed NHL. Five and 8 patients showed only either small cells or large cells in their BM, respectively. The pattern of infiltration was similarly distributed among our patients and the diffuse pattern was more frequent in large-cell categories than in mixed-cell type. Diffuse involvement and a high percentage of large cells have been reported as predicting poorer survival and FFS.[2-6] We, however, have been unable to identify any factor present at diagnosis that might predict a poor outcome in our patients with the exception of B symptoms. Perhaps this is because of the small number of patients studied. Alternatively, it might be related to the substantial CR rate, which would compensate for the influence of negative factors present at diagnosis.

In conclusion, this study suggests that intensified treatment with high-dose CY and HDT with PBPC rescue is feasible in patients with aggressive NHL with BM involvement when performed after a short course of VACOP-B chemotherapy. However, a randomized study is required to compare this strategy with conventional treatment. Such a study is currently being conducted by the *Non-Hodgkin's Lymhoma Co-operative Study Group*.

Contributions and Acknowledgments

All listed authors contributed to the conception and design, analysis and interpretation of data, to drafting the article and on final approval of the version. The order of authorship was based on the number of entered patients.

We thank Maurizia Cristanetti and Simona Barozzi for data management.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

Manuscript received June 4, 1999; accepted September 13, 1999.

Potential implications for clinical practice

- A sequential treatment of 8 weeks of VACOP-B followed first by high dose Cytoxan with G-CSF support and later by HDT is feasible and increases CR rate.
- HDCY is highly effective in reducing tumour burden.
- A randomized study is underway in order to confirm the efficacy of this strategy and compare it with conventional treatment.

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