High dose sequential chemotherapy with autologous transplantation versus dose-dense chemotherapy *MegaCEOP* as first line treatment in poor-prognosis diffuse large cell lymphoma: an "Intergruppo Italiano Linfomi" randomized trial

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Background and Objectives. Poor prognosis diffuse large cell lymphoma (DLCL) responds poorly to standard chemotherapy. Randomized studies comparing high-dose chemotherapy with autologous stem-cell transplantation (ASCT) against standard chemotherapy have produced conflicting results. Dose-dense chemotherapy with granulocyte colony-stimulating factor (G-CSF) support seems to hold promise. The purpose of this multicenter, randomized trial was to compare failure-free and overall survival in patients with poor prognosis DLCL treated with high-dose sequential (HDS) chemotherapy followed by ASCT or an outpatient dose-dense chemotherapy regimen (MegaCEOP).

Design and Methods. Between 1996 and 2001, 130 DLCL patients, aged ≤ 60 years, with intermediate-high or high-risk disease, according to the International Prognostic Index score, and/or bone marrow involvement were enrolled. Sixty were randomized to HDS chemotherapy plus high-dose mitoxantrone and melphalan with ASCT (arm A) and 66 to the MegaCEOP regimen (6-8 courses of an escalated dose of cyclophosphamide and epirubicin plus vincristine and prednisone with G-CSF every 2-weeks) (arm B); 4 patients were considered ineligible.

Results. The complete remission rate was 59% in arm A and 70% in arm B (p=0.18). After a median follow-up of 78 months, the 6-year failure-free survival was 45% in arm A and 48% in arm B (hazard ratio=1.15, 95% confidence intervals =0.72-1.84, p=0.56). The 5-year overall survival was 49% in arm A and 63% in arm B (hazard ratio=1.67, 95% confidence interval=0.98-2.85, p=0.06). Two cases of secondary acute myeloid leukemia were observed after treatment in group A.

Interpretations and Conclusions. HDS and ASCT as initial therapy for patients with poor-prognosis DLCL does not provide a benefit over that of outpatient dose-dense MegaCEOP chemotherapy.

Key words: autologous transplantation, dose-dense chemotherapy, poor-prognosis DLCL.

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Patients with diffuse large-cell lymphoma (DLCL) have a long-term cure rate with cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) of 35%[1] and third-generation regimens have failed to show better results in a large randomized clinical trial.[2] To date, patients at intermediate-high or high risk, according to the age-adjusted International Prognostic Index (IPI) score, have a long-term survival rate less than 30-35% if treated with standard chemotherapy.[3] High-dose chemotherapy with autologous stem cell transplantation (ASCT) has been proven to be an effective salvage treatment for chemosensitive relapsed patients.[4] Many investigators have extended this approach as part of the initial therapy of patients with DLCL, especially those considered at poor prognosis, with conflicting results in pilot and randomized studies.[5-10] Recently, a randomized trial performed in low-to-intermediate risk patients showed that event-free survival was better in patients treated with high-dose chemotherapy and ASCT than in those treated with CHOP.[9] In contrast, another trial in poor-prognosis aggressive lymphomas at diagnosis failed to demonstrate any benefit from high-dose chemotherapy and ASCT compared to standard chemotherapy.[10]

An alternative approach is to increase the dose and/or shorten the interval of administration of chemotherapy drugs delivered in an outpatient setting without stem cell support. The doses of cyclophosphamide and doxorubicin, or one of its analogs such as epirubicin, may be escalated and are effective in the treatment of DLCL, although the superiority of this strategy over standard chemotherapy has not yet been clearly proven.[11-14] A phase II study showed that

dose-dense chemotherapy, with increased doses of epirubicin and cyclophosphamide and shorter intervals between courses with granulocyte-colony stimulating factor (G-CSF) support (MegaCEOP), is feasible and effective in the treatment of DLCL.[14] Based on this rationale, a randomized trial was designed by the Intergruppo Italiano Linfomi to compare failure-free and overall survival between these two approaches in patients with advanced stage poor-prognosis DLCL. At diagnosis, patients were randomly allocated to receive the high-dose sequential (HDS) regimen of chemotherapy followed by ASCT support[6] or a dose-dense outpatient chemotherapy regimen, MegaCEOP, with G-CSF support.

Design and Methods

Eligibility criteria

This study was a prospective, randomized trial of the treatment of poor prognosis DLCL conducted in fourteen Italian hematology departments by the Intergruppo Italiano Linfomi (*see appendix*). From January 1996 to March 2001, 130 consecutive patients affected by DLCL entered the study. Inclusion criteria were as follows: previously untreated aggressive lymphoma (diffuse large B-cell, anaplastic or peripheral T-cell lymphoma according to the REAL classification);[15] age 18 to 60 years; 0-2 Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0-2; age-adjusted International Prognostic Index (IPI) score defined as intermediate-high (IH) and high (H) risk;[3] no central nervous system (CNS) involvement at diagnosis; normal renal, cardiac, pulmonary and hepatic function; negative serology for human immunodeficiency, hepatitis B or hepatitis C virus. Based on previous data reported by our group, patients who had large cell bone marrow (BM) involvement were also considered at poor prognosis and enrolled into the study regardless of the IPI score.[16] Central pathologic review was not routinely planned, however the histological diagnosis of 71 patients was checked at the Pathology Department of the University of Turin by DN. Details of the histological subtypes are listed in Table 1. The trial was approved by all institutions' ethics committee and all patients gave their written informed consent to participation.

Staging

Staging included physical examination, computed tomography of the chest, abdomen and pelvis, cerebral spinal fluid examination, bone marrow biopsy, blood cell counts and differential, routine blood chemistry, and MUGA scans or echocardiography. Bulky disease was defined as a mass > 10 cm in one diameter or more than one-third of the chest diameter in the mediastinum. PS and toxicity were assessed according to the ECOG scale and WHO toxicity criteria grading system, respectively.

Table 1. Initial characteristics of the patients according to treatment group: HDS + ASCT versus MegaCEOP.

Characteristic	*Arm A: HDS + ASCT (n = 60)*		*Arm B: (n = 66)*	
	No. of patients	*%*	*No. of patients*	*%*
Median age, years (range)	42 (18-59)		43 (18-60)	
Gender				
Male	35	58	37	56
Female	25	42	29	44
Histological sub-types				
Diffuse large B-cell	48	80	59	90
Primary mediastinal B-cell	6	10	4	6
Peripheral T-cell	4	7	2	3
Anaplastic large cell	2	3	1	1
B symptoms*				
Absent	18	30	30	45
Present	42	70	36	55
Performance status grade				
0-1	22	37	29	44
>1	38	63	37	56
Ann Arbor stage				
II	16	26	12	18
III	13	22	14	21
IV	31	52	40	61
No. of extranodal sites				
0-1	45	75	52	79
≥ 2	15	25	14	21
Bone marrow involvement				
Absent	47	78	48	73
Present	13	22	18	27
Tumor bulk*				
Absent	24	40	38	58
Present (>10 cm)	36	60	28	42
Serum LDH level				
< normal	15	25	17	26
≥ normal	45	75	49	74
Age-adjusted IPI risk				
Low-Intermediate	8	13	13	20
Intermediate-High	31	52	28	42
High	21	35	25	38

** The difference is statistically significant p<0.05.*

Treatment plan

Patients were centrally randomized by fax between the HDS regimen followed by ASCT[6] (arm A) and outpatient MegaCEOP chemotherapy regimen (arm B). The randomization sequence was generated by the co-ordinating center using a random-number table and stratified for participating center and BM involvement. Randomization was concealed

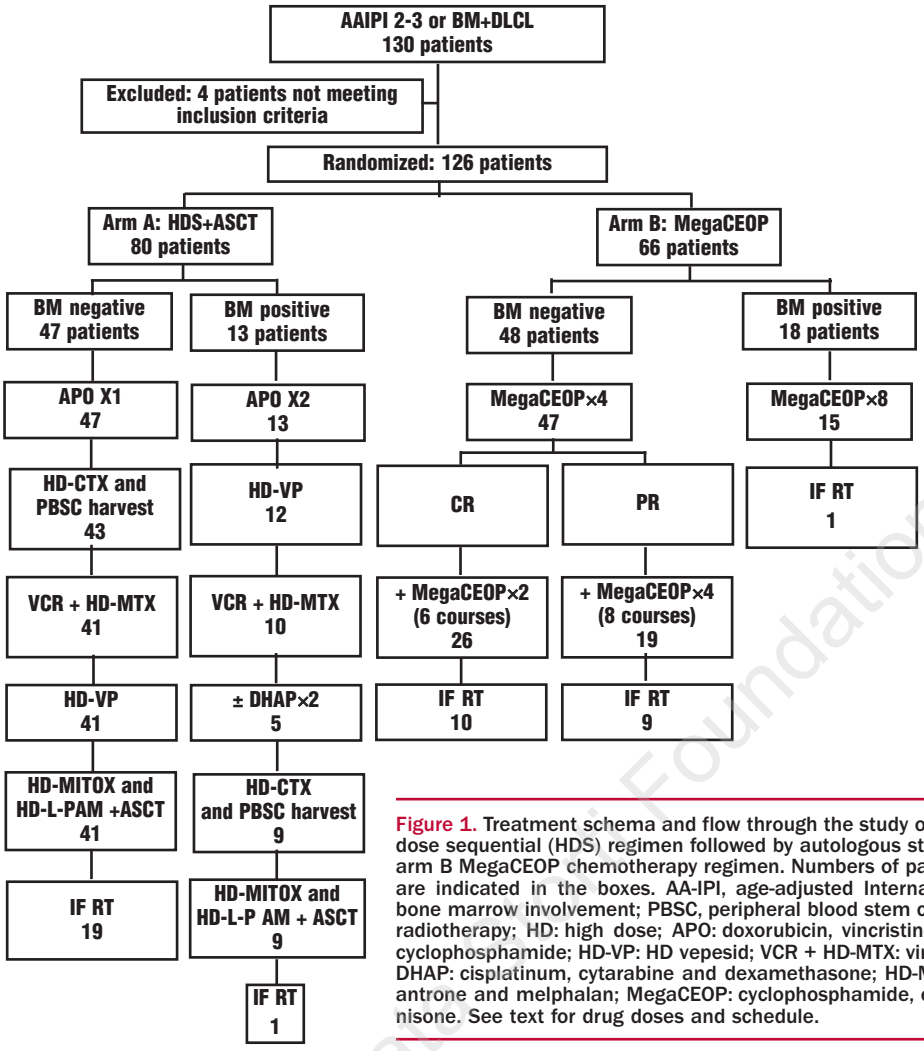


Figure 1. Treatment schema and flow through the study of the 130 patients: arm A: high dose sequential (HDS) regimen followed by autologous stem cell transplantation (ASCT); arm B MegaCEOP chemotherapy regimen. Numbers of patients at each stage of therapy are indicated in the boxes. AA-IPI, age-adjusted International Prognostic Index; BM+, bone marrow involvement; PBSC, peripheral blood stem cell harvest; IF RT, involved field radiotherapy; HD: high dose; APO: doxorubicin, vincristine and prednisone; HD-CTX: HD cyclophosphamide; HD-VP: HD vepesid; VCR + HD-MTX: vincristine plus HD methotrexate; DHAP: cisplatinum, cytarabine and dexamethasone; HD-Mitox and HD-L-PAM: HD mitoxantrone and melphalan; MegaCEOP: cyclophosphamide, epirubicin, vincristine and prednisone. See text for drug doses and schedule.

until the interventions were assigned. The trial design is shown in Figure 1. Arm A consisted in three phases: 1) doxorubicin 50 mg/m^2 on day 1, vincristine 1.4 mg/m^2 (maximum 2 mg) on days 1, 8 and 15, prednisone 40 mg/m^2 from day 1 to 21 (APO regimen); 2) sequential administration, at 15-21 day intervals, of three single high-dose chemotherapy courses with cyclophosphamide 7 g/m^2 plus G-CSF 5 μg/Kg/day (Filgrastim, Amgen/Roche, Milan, Italy) from day 2 until the peripheral-blood stem cell (PBSC) harvest, methotrexate 8 g/m^2 + vincristine 1.4 mg/m^2 (maximum 2 mg) with leucovorin rescue and etoposide 2 g/m^2 plus G-CSF 5 μg/Kg/day from day 4 until recovery of neutrophil counts; 3) mitoxantrone 60 mg/m^2 on day – 5 and melphalan 180 mg/m^2 on day – 2 followed by ASCT. Patients with BM involvement were given a modified HDS regimen as follows: two courses of APO; an inverted sequence with methotrexate, etoposide, two additional courses of DHAP[17] in case of less than partial response or persistence of BM involvement and cyclophosphamide + G-CSF with PBSC harvest. Patients randomized to arm A with no response after the HDS regimen or progressive disease at any time did not proceed to ASCT.

Arm B consisted in epirubicin 110 mg/m^2 on day 1, cyclophosphamide 1200 mg/m^2 on day 1, vincristine 1.4 mg/m^2 (maximum 2 mg) on day 1, and prednisone 40 mg/m^2 from day 1 to 5 at 2-week intervals.[14] G-CSF support (5 μg/Kg/day) was given from day 2 to day 10. In case of neutropenia < 1.5×0^9/L chemotherapy was delayed for one week. No dose reduction or delays were allowed in the case of thrombocytopenia, but platelet transfusions were given if the platelet count was <15×0^9/L. Patients in complete remission after four courses of MegaCEOP chemotherapy received two additional courses, those in partial remission or stable disease were given four additional courses. Patients with BM involvement always received eight courses of

MegaCEOP. Patients with BM, testicular or paranasal sinus involvement were given CNS prophylaxis with intrathecal methotrexate 12 mg/m^2; four doses were given in arm A and six doses in arm B. At the end of the treatment, radiotherapy (36 Gy) was given to areas of previous bulky disease.

Stem cell harvesting

At least 3×10^6/Kg peripheral blood CD34$^+$ cells were collected after cyclophosphamide. In the case of inadequate PBSC collection, stem cells were harvested from the BM.

Supportive care

Patients randomized to group A were given the entire program in standard, non-protected rooms. Patients randomized to group B received their treatment in an outpatient setting.

Assessment of response

An intermediate assessment of response was planned in each arm. In arm A patients were restaged before ASCT. Those with BM involvement underwent an additional earlier revaluation, including BM biopsy, after high dose etoposide in order to decide-whether or not to administer two additional courses of DHAP. In arm B patients were revaluated after four courses of the MegaCEOP regimen. In both arms, the final response assessment was performed one month after the end of the whole therapeutic program. Complete remission (CR) was defined as the absence of any detectable clinical and radiographic disease with disappearance of all disease-related symptoms. Unconfirmed complete remission (CRu) was considered as a persistent clinical or radiographic lymph-node mass that regressed by more than 75% of the initial tumor volume and no signs or symptoms of active disease. If the radiological abnormalities were subsequently stable for at least three months the patients were judged to have a CR. Patients with a 50% or greater reduction in tumor volume were considered in partial remission (PR). No response was defined as anything less than a PR or progressive disease or any death during the treatment period.[18]

Study design and statistical methods

The primary endpoint of the study was failure-free survival (FFS), with failure defined as an incomplete response, relapse or death from any cause and was used to determine the sample size of the study. The study was designed according to data on the HDS regimen available in 1996 that showed a clear advantage for FFS in favor of high-dose chemotherapy with ASCT compared to standard chemotherapy.[6] The main hypothesis of the study was to confirm these results and detect an increase of 25% in the probability of FFS at 3 years in favor of the HDS+ASCT arm. With a two-sided α error of 0.05 and a β error of 0.20 and assuming a 50% 3-year FFS in the MegaCEOP arm versus an expected 75% in the HDS+ASCT arm, this design required the randomization of 62 patients per arm and an estimated time of about 5 years to reach these numbers. Secondary endpoints were overall survival (OS) and clinical response.

All the randomized patients were considered assessable and results were analyzed on an intention-to-treat basis. Analysis was based on disease status in November 2004 with a minimum of 3 years of follow-up. All survival and failure-free times were censored at the closing date or the date of last contact, whichever came first. The FFS analysis included all patients and was calculated from the date of diagnosis to the date of incomplete response, relapse, progression or death from any cause or last follow-up without any event. The OS analysis included all patients, and death due to any cause; OS was measured from the date of diagnosis to the date of death or last follow-up alive. Disease-free survival (DFS) was applied only to patients who achieved a CR/Cru and was calculated from the time of CR/CRu assessment to the date of relapse or last follow-up free of disease. All curves were plotted according to the method of Kaplan and Meier[19] and statistical differences among curves were evaluated by the log rank test. The Cox proportional hazard model was used to estimate hazard ratios (HR) and corresponding 95% confidence intervals (95% CI), adjusted for unbalanced or important prognostic factors (BM involvement, IPI score).[20]

The proportions of responses and toxic effects were compared between the two arms by a χ^2 test or Fisher's exact test, depending on the size of the sample evaluated. Means were compared by two sided t-tests. All calculations were done using the SAS (v. 8.2) package.

The data were collected by one investigator at each participating center, sent to the centralized database in Turin and checked for accuracy by hematology research assistants at the University of Turin.

Results

Patients' characteristics

One hundred and thirty patients were randomized. During the first cycle of therapy four patients, two in each arm, were found to violate the entry criteria and were therefore excluded. Of these, two had CNS involvement at diagnosis and two had Burkitt's lymphoma at histological revision. Thus 126 patients were considered eligible: 60 were randomized to

receive HDS + ASCT (arm A) and 66 MegaCEOP chemotherapy (arm B). The clinical characteristics of the 126 patients are listed in Table 1 and were well balanced between the two arms, except for an excess of patients with bulky disease and B symptoms in the HDS+ASCT group.

Feasibility

Overall 110 (87%) patients completed the assigned treatment. Fifty-nine of 64 patients who had bulky disease at diagnosis completed the chemotherapy treatment. Consolidation radiotherapy to previous bulky areas was delivered to patients who completed the chemotherapy: 20 of 32 (62%) patients with bulky disease in arm A and 20 of the 27 (74%) patients in arm B (p=0.34).

Arm A. Fifty (83%) of the 60 patients completed the treatment and underwent ASCT; the source of stem cells was peripheral blood in 47 patients and autologous BM cells in the other 3 patients. Reasons for not completing the planned treatment in 10 patients were: disease progression before ASCT in 8 patients, acute congestive heart failure after two courses of APO in one and toxic death in one. Five patients with BM involvement received two additional courses of DHAP because of persistence of BM involvement or less than PR after high dose etoposide.

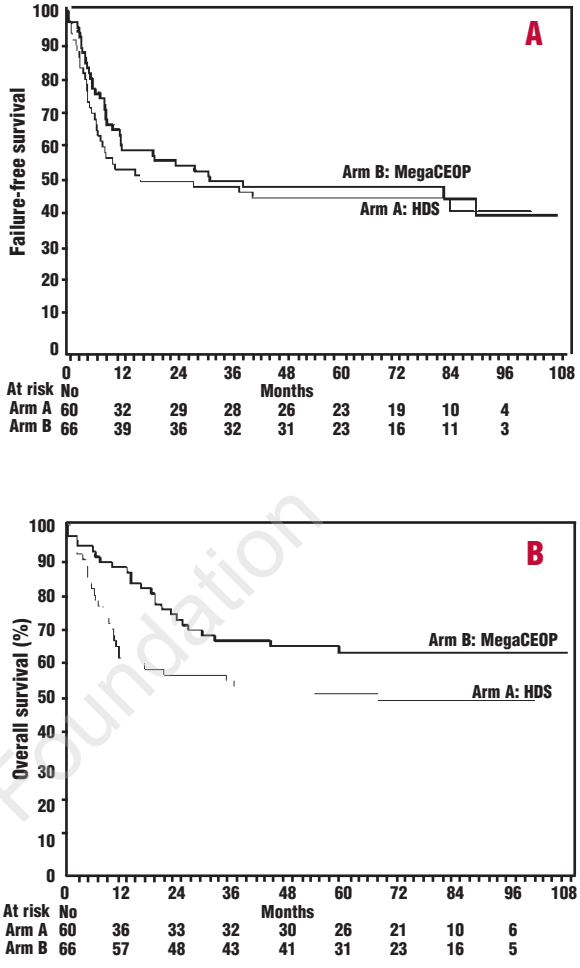
Arm B. Four hundred and forty-one courses of MegaCEOP chemotherapy were delivered with a mean of 6.7 courses per patient. Sixty (92%) of 66 patients completed the planned MegaCEOP chemotherapy. Six interrupted the treatment: 4 because of progressive disease and 2 because of toxic deaths.

Response to treatment and outcome

Thirty-five (59%) patients achieved CR + CRu in arm A and 46 (70%) in arm B (p=0.18). Partial remission was documented in 6 (10%) patients in arm A and in 6 (9%) in arm B, while during treatment 17 (28%) patients in arm A and 12 (18%) in arm B progressed. Two patients in each arm (3%) died of toxicity.

After a median follow up of 78 months, treatment had failed in 70 patients (arm A 34, arm B 36) and 55 died (arm A 31, arm B 24). There was no statistically significant difference in 5-year FFS between the two groups: 45% for arm A and 48% for arm B, with a crude hazard ratio (HR)=1.15 (95% CI=0.72-1.84, p=0.56) (Figure 2A).

There was a trend for a lower 6-year overall survival rate in arm A (49%) than in arm B (63%), with a crude HR=1.67 (95% CI=0.98-2.85, p=0.06) (Figure 2B). Relapses were observed in 9 of the 35 patients who had achieved CR in arm A and in 16 of the 46 with CR in arm B, thus the 6-year DFS rates were



Figures 2A, B. 6-year FFS (a) and OS (b) according to the treatment arm. Arm A (HDS + ASCT); Arm B (MegaCEOP). A. FFS at six years: 45% (Arm A) vs. 48% (Arm B) p=0.52 B. OS at six years: 49% (Arm A) vs. 63% (Arm B) p=0.06.

72% in arm A and 69% in arm B, with a crude HR=0.69 (95% CI=0.31-1.56, p=0.37). A Cox's model was performed to adjust the effect of the treatment for potential confounders such as B symptoms and bulky disease, included into the model due to their unbalanced distribution between the two arms, and BM involvement and age-adjusted IPI score, included as main prognostic factors. In this multivariate analysis, FFS was not affected by treatment, with an adjusted HR (arm A vs. arm B)= 1.17 (95% CI=0.72-1.89, p=0.53), while a higher age-adjusted IPI score showed a tendency to increase the failure risk, with an adjusted HR (for each point of IPI score)=1.38 (95% CI 0.97–1.97, p=0.07).

In the multivariate analysis overall survival was marginally affected by treatment, with an adjusted HR (arm A vs. arm B)=1.67 (95% CI 0.96 - 2.90, p=0.07). The age-adjusted IPI score was a strong pre-

Table 2. WHO grade 3-4 toxicity: HDS + ASCT versus MegaCEOP.

Type of toxicity	Arm A: HDS + ASCT (n = 60)				Arm B: MegaCEOP (n = 66)	
	HDS (n = 60)	ASCT (n = 50)	HDS + ASCT* (n = 60) No of pts.	%	No of pts.	%
Severe infections	9	11	17	28	6	9
Mucositis	4	18	22	37	2	3
Cardiac	2	1	3	5	1	2
Neurological	2	1	3	5	3	5
Gastro-intestinal	5	4	9	15	5	8
Renal	1	1	2	3	1	2
Acute toxic deaths	1	1	2	3	2	3
Secondary MDS/ANLL	_	_	2	3	0	0

N. and percentage of patients who showed grade 3-4 toxicity in HDS or ASCT phase.

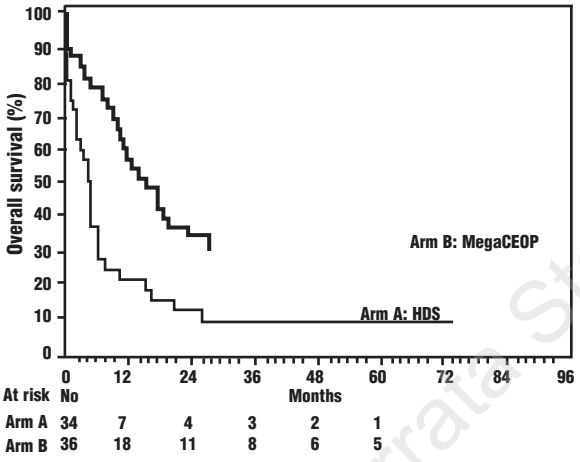


Figure 3. OS of patients who relapsed or had disease progression and had been treated with HDS+ASCT or MegaCEOP. OS was calculated from the date of relapse or progression. OS at two years: 12% (Arm A) vs. 33% (Arm B) p=0.0015.

dictor of death: adjusted HR (for each point of IPI score)=1.83 (95% CI 1.20 - 2.79, p=0.005). A not prespecified subgroup analysis for patients with intermediate-high and high risk scores (arm A, n = 52; vs. arm B, n=53) did not reveal any statistically significant differences between the two treatment arms: FFS 44% vs. 45% (adjusted HR=1.16, 95% CI=0.69-1.98, p=0.57) and OS 48% vs. 58% (adjusted HR=1.59, 95% CI=0.89-2.85, p=0.12). No differences in 5-year FFS and OS were observed between the two treatment arms in patients with or without BM involvement (*data not shown*).

Failures and relapses

Treatment failed in 34 patients in arm A and 36 in arm B. Patients who did not respond or relapsed after remission received different salvage chemotherapy regimens. Of the 34 patients in arm A in whom treatment failed, 27 died of progressive disease, 2 died of toxicity during primary treatment, 2 died of secondary acute non-lymphoblastic leukemia (ANLL) and 3 are alive. Treatment failed in 36 patients in arm B: 22 died of progressive lymphoma, 2 of toxicity during primary treatment and 12 are alive. The 2-year survival from the date of relapse or progression was significantly lower for arm A (12%) than for arm B (33%), with a crude HR=2.41 (95% CI 1.40-4.15, p=0.0015) (Figure 3).

Toxicity

All 50 patients who underwent ASCT with high dose mitoxantrone and melphalan in arm A achieved complete hematologic engraftment with neutrophil counts > 0.5×10^9/L after a median of 11 days (range, 6 to 30 days) and a self-sustaining platelet count > 50×10^9/L after a median of 12 days (range, 5 to 98 days). Twenty-seven per cent required packed red cell transfusions during the HDS regimen and 86% after ASCT. Platelet concentrates were given to 18% of the patients during the HDS regimen and 88% after ASCT.

MegaCEOP chemotherapy in arm B was delivered in an outpatient setting. In this study group grade 4 hematologic toxicity for neutrophils, platelets and hemoglobin was recorded in 22%, 2% and 1.4% of the total delivered courses and occurred in 48%, 3% and 1.5% of the patients, respectively. Packed red cell transfusions and platelet concentrates were given to 43% and 6% of the patients, respectively.

There were four toxic deaths, all infection-related, two in each arm. In arm A, one patient died of pulmonary aspergillosis after APO chemotherapy and the second one died of neutropenic sepsis after ASCT. In arm B two fatal infections (neutropenic sepsis and a bilateral bacterial pneumonia) occurred during treatment. Severe non-hematologic toxicities, WHO grade 3 or 4, are reported in Table 2.

Two cases of secondary ANLL or myelodysplastic syndrome (MDS) occurred at 23 and 30 months after ASCT in arm A. Both patients were in CR of their lymphoma and died of secondary leukemia.

Discussion

The aim of this Intergruppo Italiano Linfomi randomized trial was to assess the potential benefit of a HDS chemotherapy regimen with ASCT support com-

pared to a dose-dense outpatient MegaCEOP chemotherapy regimen with G-CSF support in patients with *de novo* poor prognosis aggressive lymphoma. Unlike some other trials dealing with this issue, patients were randomized up-front between two completely different regimens in order to properly evaluate the role of high dose chemotherapy with ASCT as initial treatment. The present study failed to show any advantage of HDS and ASCT in this group of patients and acute toxicities such as mucositis and infections were reported less frequently in the MegaCEOP arm. The CR rate was higher, although not to a statistically significant extent (70% vs. 59%), in patients treated with MegaCEOP chemotherapy, which is intensively delivered every two weeks. No differences in relapse rates were observed and the 6-year actuarial FFS rates (45% vs. 48%) were not different between the two arms. The results of the adjusted comparisons in the Cox's model for FFS confirmed the lack of difference in the outcome between HDS+ASCT and MegaCEOP chemotherapy, excluding a confounding effect of a slightly unbalanced distribution between groups of a few prognostic variables. Our trial protocol allowed the inclusion of patients at low-intermediate risk if they had large cell BM involvement; however, after excluding this small group of patients (17%), the results did not change. Moreover there was a strong suggestion that patients treated with MegaCEOP chemotherapy have a more prolonged overall survival than those who receive HDS and ASCT (63% vs. 49%) (p=0.06). This may be due to a more effective salvage therapy for patients who failed MegaCEOP chemotherapy, leading to a 2-year survival rate from progression or relapse of 33% compared to only 12% for patients in whom HDS failed. Similar data from other randomized studies were reported and underscore the extreme difficulty of effectively salvaging high-risk patients after high-dose chemotherapy has failed.[10,21]

Some issues must be pointed out in order to explain the results of the present trial. Firstly, 17% of the patients in arm A did not receive ASCT, whereas in arm B only 9% of patients interrupted the planned MegaCEOP chemotherapy. The difficulty in delivering high-dose chemotherapy with ASCT has been reported in other randomized studies and is in the range between 26% and 40%.[8,10,21,22]

It is noteworthy that in both arms of our study the main reason for not completing therapy was induction failure, which occurred more frequently in the transplantation arm. Secondly, the results obtained in arm A with the HDS chemotherapy regimen were worse than expected given previously published data on the same treatment, although the HDS regimen was applied exactly as described.[6] Our study was prospectively designed for poor prognosis patients who had either an intermediate-high or high risk IPI score or BM involvement at the time of diagnosis. Patients who met these criteria at the participating centers were enrolled and analyses were performed on an intention-to-treat basis, whereas in the trial by Gianni *et al*.[6] IPI was determined retrospectively, thus the patient populations may be different and not limited to patients with a poor prognosis.

A number of trials have been conducted to evaluate the benefit of high-dose chemotherapy with ASCT as first line treatment in aggressive lymphomas, but controversy still exists regarding the impact of this strategy. Differences in inclusion criteria, selection not always based on the IPI score, various drugs schedules and different durations of the pre-ASCT chemotherapy phase could be responsible for the discrepancies reported.[6,8,21-22] Recently, Milpied *et al*.[9] showed an advantage in terms of event-free survival for high-dose chemotherapy with ASCT compared to standard CHOP in a randomized cohort of 207 patients with aggressive lymphoma. However the advantage was limited to low-to-intermediate risk patients because high-risk patients were excluded from the trial and the high-dose chemotherapy started with two courses of a dose-dense CEEP regimen that might have contributed to the better outcome. In another trial by the GELA group, 370 patients were randomized at diagnosis to an abbreviated course of induction chemotherapy followed by BEAM and ASCT or four courses of intensified chemotherapy with ACVB at 2-week intervals with filgrastim support, followed by consolidation with standard chemotherapy.[10] Interestingly, this study which employed ACVB, a regimen similar to the MegaCEOP used in our trial, demonstrated a benefit in term of overall and event-free survival (EFS) for patients treated with the ACVB regimen.

An outpatient dose-dense CHOP-like regimen with G-CSF support may be an alternative strategy to high-dose chemotherapy in patients with poor-risk aggressive lymphoma and promising results have been reported in phase II studies.[11-14,23-24] On the other hand a prospective trial failed to show that increasing dose intensity influences outcome.[25] Recently, three randomized trials showed that increased doxorubicin and cyclophosphamide doses or decreased intervals between cycles could improve treatment results.[26-28] In a GELA trial, the ACVB regimen was reported to produce a better 5-year EFS and OS compared to standard CHOP chemotherapy, although this study was limited to elderly patients and also included patients at low-intermediate risk.[26] The German Lymphoma Study Group reported that the addition of etoposide to CHOP, mainly at decreased intervals (CHOEP-14), improved CR, 5-year EFS and OS compared to CHOP-21 in young patients with aggressive lymphomas and a

good prognosis (normal lactate dehydrogenase levels) and shortening the intervals between cycles (CHOP-14) improved 5-year EFS and OS in elderly patients.[27-28] However dose-dense chemotherapy regimens may have more acute or long-term side effects as reported by some authors.[11,26] Thus, this strategy may be as effective as high-dose chemotherapy with ASCT, but its potential benefit over standard chemotherapy remains to be clearly demonstrated, particularly in young patients at intermediate-high or high risk. In the present study, two patients treated with HDS chemotherapy and ASCT developed a secondary ANLL or MDS. Although this figure is in the range of those reported after high-dose chemotherapy, its occurrence in patients CR is worrisome.[29]

One important limit of our study should be highlighted. Our trial was designed to detect a 25% increase of probability of 3-year FFS in favor of the transplantation arm, based on previous data with the HDS + ASCT regimen reported by Gianni *et al.* from 98 patients who showed an event-free survival rate of 76% for HDC with ASCT compared to 49% for standard chemotherapy.[6] The sample size of the present trial was calculated with a two-sided test based on these expected figures. However the results of other trials that were reported in the following years, when our study was ongoing, did not confirm these excellent results in favor of high-dose chemotherapy. Thus our trial was clearly underpowered and unable to detect smaller differences between treatment arms, such as the suggested survival advantage among recipients of the dose-dense chemotherapy, which might have been evident if the study had been designed for a smaller difference in a larger cohort of patients.

The proper role and place of high-dose chemotherapy and ASCT as part of first line treatment in poor risk DLCL remains a controversial issue. Increasing the CR rate remains the main aim to improve the outcome of patients with poor prognosis aggressive lymphoma. The addition of rituximab has proven to increase the effectiveness of standard CHOP chemotherapy significantly and may reduce lymphoma cell contamination from stem cell harvests as an *in vivo* purging agent before ASCT.[30-31] High-dose chemotherapy with ASCT might be better at prolonging remission duration in CR patients at high and intermediate-high risk when this strategy is used after maximum tumor reduction has been achieved with a full course of chemotherapy.[32-33] However, in order to avoid over-treatment and long-term morbidity, its value as up-front therapy needs to be weighed against standard or dose-dense outpatient chemotherapy in combination with anti-CD20 as first line treatment, with the option of high-dose chemotherapy and ASCT as salvage therapy in cases of failure.[4] A dose-dense outpatient chemotherapy regimen, likely with concurrent rituximab, may be a feasible and effective alternative approach to improve the prognosis of poor risk DLCL.

Appendix

List of the participating institutions and physicians of the Intergruppo Italiano Linfomi.

S.C. Ematologia, ASO SS Antonio e Biagio, Alessandria: Flavia Salvi, Alessandro Levis; Cattedra di Ematologia, Università di Bari: Enzo Pavone, Vincenzo Liso; S.C. Ematologia e Centro Trapianto Midollo Osseo, Ospedale Oncologico "Armando Businco, Cagliari: Maria Giuseppina Cabras, Angela Maria Mamusa, Emanuele Angelucci; S.C. Ematologia, Ospedale Papardo, Messina and S.C. Emato-Oncologia, ASO Bianchi-Melacrino-Morelli, Reggio Calabria: Fortunato Morabito, Francesco Nobile, Maura Brugiatelli; Dipartimento di Ematologia, Ospedale Maggioe IRCCS Milano: Luca Baldini, M Goldaniga, AT Maiolo, Giorgio Lambertenghi Deliliers; Oncologia Medica, Università di Modena e Reggio Emilia: Massimo Federico; Dipartimento di Scienze Cliniche e Biologiche, ASO S.Luigi e Università di Torino, Orbassano: Guido Parvis, Giuseppe Saglio; Istituto di Medicina Interna e Scienze Oncologiche, Perugia: Paola degli Angeli, Alessandro Genua, Mario Trottini, Marina Liberati; S.C. Ematologia Clinica, Presidio Ospedaliero dello Spirito Santo, Pescara: Marco Sborgia, Giuseppe Fioritoni; Cattedra di Ematologia, Università di Pisa: Francesco Caracciolo, Mario Petrini; S.C. Ematologia, ASO San Carlo, Potenza: Michele Pizzuti, Francesco Ricciuti; S.C. Ematologia, Casa Sollievo della Sofferenza, S.Giovanni Rotondo: Nicola Di Renzo, Angelo Maria Carella; S.C. Ematologia ASO S.Giovanni Battista, Torino: Carola Boccomini, Roberta Calvi, Roberto Freilone, Lorella Orsucci, Umberto Vitolo, Eugenio Gallo; Cattedra di Ematologia, Università di Verona: Giuseppe Todeschini, Giovanni Pizzolo.

While all the authors fulfill the requirements for authorship more specifically: UV conceived and designed the trial, interpreted the data and wrote the manuscript; AML, EA, GLD, AL, EG trial design, data interpretation and manuscript revision; GC statistical design and data analysis; RC, CB data collection and data accuracy check; MGC, MF, MB patient accrual, data interpretation and manuscript revision; LB, AG, GP, EP, FS, MS patient accrual and monitoring, data collection. The authors declare that they have no potential conflicts of interest.

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References

1. Coltman AC Jr, Dahlberg S, Jones ES, et al. CHOP is curative in thirty per cent of patients with large cell lymphoma: a twelve year Southwest Oncology Group follow-up, in Skarin AT, Editor. Advances in Cancer Chemotherapy: Update on Treatment for Diffuse Large Cell Lymphoma. New York, NY; Park Row 1986. p. 71-7.
2. Fisher RI, Gaynor ER, Dahlberg R, Oken MM, Grogan TM, Mize EM, et al. Comparison of standard regimen (CHOP) with three intensive chemotherapy regimens for advanced non-Hodgkin's lymphoma. N Engl J Med 1993;328:1002-6.
3. Shipp MA, Harrington DP. A predictive model for aggressive NHL: the International Non-Hodgkin's Lymphoma Prognostic Factor Project. N Engl J Med 1993;329:987-94.
4. Philip T, Guglielmi C, Hagenbeek A, Somers R, Van der Lelie H, Bron D, et al. Autologous bone marrow transplantation as compared with salvage chemotherapy in relapses of chemotherapy-sensitive non-Hodgkin's lymphoma. N Engl J Med 1995;333:1540-5.
5. Vitolo U, Cortellazzo S, Liberati AM, Freilone R, Falda M, Bertini M, et al. Intensified and high dose chemotherapy with granulocyte colony-stimulating factor and autologous stem-cell transplantation support as first-line therapy in high-risk diffuse large-cell lymphoma. J Clin Oncol 1997;15:491-8.
6. Gianni AM, Bregni M, Siena S, Brambilla C, Di Nicola M, Lombardi F, et al. High dose chemotherapy and autologous bone marrow transplantation compared with MACOP-B in aggressive B-cell lymphoma. N Engl J Med 1997;336:1290-7.
7. Santini G, Salvagno L, Leoni P, Chisesi T, De Souza C, Sertoli MR, et al. VACOP-B versus VACOP-B plus autologous bone marrow transplantation for advanced diffuse non Hodgkin's lymphoma: results of a prospective randomized trial by the non Hodgkin's Lymphoma Cooperative Study Group. J Clin Oncol 1998;16:2796-802.
8. Haioun C, Lepage E, Gisselbrecht C, Salles G, Coiffier B, Brice P, et al. Survival benefit of high dose therapy in poor risk non Hodgkin's lymphoma: final analysis of the prospective LNH87-2 protocol, a groupe d'Etude des lymhomes de l'Adulte study. J Clin Oncol 2000;18:3025-30.
9. Milpied N, Decorininck E, Gaillard F, Delwail V, Foussard C, Berthou C, et al. Initial treatment of aggressive lymphoma with high-dose chemotherapy and autologous stem-cell support. Groupe Ouest-Est des Leucemies et des Autres Maladies du Sang. N Engl J Med 2004; 350:1287-95.
10. Gisselbrecht C, Lepage E, Molina T, Quesnel B, Fillet G, Lederlin P, et al. Shortened first-line high dose chemotherapy for patients with poor-prognosis aggressive lymphoma. Groupe d'Etude des Lymphomes de l'Adulte. J Clin Oncol 2002;20:2472-9.
11. Shipp MA, Neuberg D, Janicek M, Canellos GP, Shulman LN. High-dose CHOP as initial therapy for patients with poor-prognosis aggressive non-Hodgkin's lymphoma: a dose-finding pilot study. J Clin Oncol 1995;13:2916-23.
12. Tilly H, Mounier N, Lederlin P, Briere J, Dupriez B, Sebban C, et al. Randomized comparison of ACVBP and m-BACOD in the treatment of patients with low-risk aggressive lymphoma: the LNH87-1 study. J Clin Oncol 2000; 18:1309-15.
13. Talbot SM, Westerman DA, Grigg AP, Toner GC, Wolf M, Bishop J, et al. Phase I and subsequent phase II study of filgrastim (r-met-HuG-CSF) and dose intensified cyclophosphamide plus epirubicine in patients with non-Hodgkin's lymphoma and advanced solid tumors. Ann Oncol 1999;10:907-14.
14. Vitolo U, Botto B, Boccomini C. Intensified chemotherapy with dose intensity escalation of cyclophosphamide and epirubicin with filgrastim support (MegaCEOP) for poor prognosis aggressive non-Hodgkin's lymphoma. Blood 1997;90 Suppl 1: 190a[Abstract].
15. Harris NL, Jaffe ES, Stein H, Banks PM, Chan JK, Cleary ML, et al. A Revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. Blood 1994;84:1361-92.
16. Vitolo U, Bertini M, Brusamolino E, Cavallero GB, Comotti B, Gallo E, et al. MACOP-B treatment in diffuse large-cell lymphoma: identification of prognostic groups in an Italian multicenter study. J Clin Oncol 1992;10: 219-27.
17. Velasquez WS, Cabanillas F, Salvador P, McLaughlin P, Fridrik M, Tucker S, et al. Effective salvage therapy for lymphoma with cisplatin in combination with high-dose Ara-C and dexamethasone (DHAP). Blood 1988;71:117-22.
18. Cheson BD, Horning SJ, Coiffier B. Report of an International Workshop to standardize response criteria for non Hodgkin's lymphomas. J Clin Oncol 1999;17:1244-53.
19. Kaplan EL, Meier P. Nonparametric estimation from incomplete information. J Am Stat Assoc 1958;53:547-81.
20. Cox DR. Regression model and life tables (with discussion). JR Stat Soc 1972;34:187-222.
21. Kaiser U, Uebelacker I, Abel U, Birkmann J, Trumper L, Schmalenberg H, et al. Randomized study to evaluate the use of high-dose therapy a spart of primary treatment for "Aggressive" lymphoma. J Clin Oncol 2002; 20:4413-9.
22. Martelli M, Gherlinzoni F, De Renzo A, Zinzani PL, De Vivo A, Cantonetti M, et al. Early autologous stem-cell transplantation versus conventional chemotherapy as front-line therapy in high-risk, aggressive non-Hodgkin's lymphoma: an Italian multicenter randomised trial. J Clin Oncol 2003;21: 1255-62.
23. Blayney DW, LeBlanc ML, Grogan T, Gaynor ER, Chapman RA, Spiridonidis CH, et al. Dose intense, every two week chemotherapy (CHOP-DI) may improve survival in intermediate and high grade lymphoma. The Southwest Oncology Group. J Clin Oncol 2003;21:2466-73.
24. Balzarotti M, Spina M, Sarina B, Magagnoli M, Castagna L, Milan I, et al. Intensified CHOP regimen in aggressive lymphomas: maximal dose intensity and dose density of doxorubicine and cyclophosphamide. Ann Oncol 2002;13:1341-6.
25. Meyer RM, Quirt IC, Skillings JR, Cripps MC, Bramwell VH, Weinerman BH, et al. Escalated as compared with standard doses of doxorubicin in BACOP therapy for patients with non-Hodgkin's lymphoma. N Engl J Med 1993;329: 1770-6.
26. Tilly H, Lepage E, Coiffier B, Blanc M, Herbrecht R, Bosly A, et al. Intensive conventional chemotherapy (ACVBP regimen) compared with standard CHOP for poor-prognosis aggressive non-Hodgkin's lymphoma. Groupe d'Etude des Lymphomes de l'Adulte. Blood 2003;102:4284-9.
27. Pfreundschuh M, Trumper L, Kloess M, Schmits R, Feller AC, Rube C, et al. Two-weekly or 3-weekly CHOP chemotherapy with or without etoposide for the treatment of young patients with good-prognosis (normal LDH) aggressive lymphomas: results of the NHL-B1 trial of the DSHNHL. Blood 2004;104:626-33.
28. Pfreundschuh M, Trümper L, Kloess M, Schmits R, Feller AC, Rube C, et al. Two-weekly or 3-weekly CHOP Chemotherapy with or without etoposide for the treatment of elderly patients with aggressive lymphomas: results of the NHL-B2 trial of the DSHNHL. German High-Grade Non-Hodgkin's Lymphoma Study Group. Blood 2004;104:634-41.
29. Milligan DW, Ruiz De Elvira MC, Kolb HJ, Goldstone AH, Meloni G, Rohatiner AZ, et al. Secondary leukemia and myelodisplasia after autografting for lymphoma: results from the EBMT lymphoma and late effects working parties. B J Haematol 1999;106:1020-6.
30. Coiffier B, Lepage E, Brière J, Herbrecht R, Tilly H, Bouabdallah R, et al. CHOP plus Rituximab with CHOP chemotherapy in elderly patients with diffuse large B-cell lymphoma: a Group d'Etude des Lymphomes de l'Adulte study. N Engl J Med 2002; 346: 235-42.
31. Gianni AM, Magni M, Martelli M, Di Nicola M, Carlo-Stella C, Pilotti S, et al. Long-term remission in mantle cell lymphoma following high-dose sequential chemotherapy and in vivo purged stem cell autografting (R-HDS regimen). Blood 2003;102:749-55.
32. Strehl J, Mey U, Glasmacher A, Djulbegovic B, Mayr C, Gorschluter M, et al. High-dose chemotherapy followed by autologous stem cell transplantation as first-line therapy in aggressive non-Hodgkin's lymphoma: a meta-analysis. Haematologica 2003; 88: 1304-15.
33. Mounier N, Gisselbrecht C, Brière J, Haioun C, Feugier P, Offner F, et al. All aggressive lymphoma subtypes do not share similar outcome after front-line autotransplantation: a matched-control analysys by the Groupe d'Etude des lymhomes de l'Adulte (GELA). Ann Oncol 2004;15:1790-7.