Background and Objectives. Therapy for relapsed/ refractory lymphomas should be based only on drugs not included in the front-line chemotherapy regimens. We adopted the strategy of using salvage chemotherapy to debulk disease and simultaneously mobilize stem cells, using a regimen based on ifosfamide and etoposide, (drugs not usually used for front-line treatment).

Design and Methods. A three-drug combination of ifosfamide, epirubicin and etoposide (IEV) was used to treat 62 patients with relapsing or refractory aggressive non-Hodgkin’s lymphoma (NHL; n=51) or Hodgkin’s disease (HD; n=11). Forty-five of the patients were studied for the feasibility of peripheral blood stem cell (PBSC) harvest.

Results. The overall and complete response (CR) rates were, respectively, 77% and 32% in the NHL subset and 81% and 45% in the HD subset. Among the 17 patients who achieved CR after IEV but did not have a subsequent transplantation, the median duration of the response was 9 months (range, 2 to 14 months). Mobilization was successful in 33 of 45 (71%) patients. Among the 45 who proceeded to autotransplantation, 27 (60%) were in CR status after the autograft; 23/45 (51%) patients are currently in continuous CR with a median follow-up of 25 months (range, 10-68 months); the relapse-free survival curve shows 83% in this state at 60 months. Twenty-three (37%) patients are currently in continuous CR with a median follow-up of 25 months. Clinical and hematologic toxic effects were mild.

Interpretation and Conclusions. Our results indicate the efficacy of the IEV regimen in inducing a good remission rate. IEV is a predictable and highly effective mobilization regimen in relapsed/ refractory patients with aggressive NHL or HD.

Malignant Lymphomas

Although advanced stage Hodgkin’s disease (HD) and some aggressive non-Hodgkin’s lymphomas (NHL) are potentially curable with standard chemotherapy, many patients either relapse or never achieve remission. One way of improving this situation could be to intensify front-line chemotherapy, either by dose-escalation of conventional therapy or by adding high-dose chemotherapy with peripheral blood stem cell (PBSC) rescue. Whereas treatment of relapsing disease with conventional chemotherapy is unsatisfactory, especially in those patients who have had an early relapse, high-dose chemotherapy and stem cell rescue may improve prognosis. Although high-dose chemotherapy can also be effective as a salvage strategy for HD patients who do not respond to induction treatment, the approach fails with many refractory primary lymphomas. So, despite our current ability to cure almost half of all aggressive NHL and the majority of HD, effective second-line therapies are needed for both resistant and relapsing disease. These second-line therapies could serve both for re-induction prior to high-dose chemotherapy with PBSC rescue, and for patients who are not candidates for transplantation.

Ideally, second-line therapy should not share any mechanism of action or toxicity with the initial induction regimen. In practical terms, since most primary induction chemotherapy regimens contain anthracyclines and tend to be myelosuppressive, second-line combinations that avoid cardiac toxicity and excessive stem cell damage would be particularly useful. In multiple trials in which combination chemotherapy has been used to treat relapsed lymphoma patients following treatment with CHOP, most of the regimens have included cisplatin, methotrexate, mitoxantrone, and ifosfamide. The MINE (ifosfamide, mitoxantrone, etoposide) regimen was one of the first to include ifosfamide, and several investigators have used variants of this combination to treat patients with...
relapsed lymphomas. All these studies have indicated that ifosfamide-based regimens incorporating etoposide and other agents can induce responses in transplanted patients and, when used in high doses in combination with growth factors, can act as excellent mobilizers of PBSC.

We previously reported on the use of IEV (ifosfamide, epirubicin, and etoposide) alone in a group of resistant or relapsing lymphoma (HD and aggressive NHL) patients. Herein, we report an extended follow-up of a larger series of patients treated with IEV either alone or prior to autologous bone marrow transplantation (ABMT).

Design and Methods

Patients' characteristics

The present study regards 62 consecutive, previously treated patients (37 males, 25 females; median age, 42 years, range 14-62) with either NHL (n=51) or HD (n=11) diagnosed between January 1995 and September 2000 (Table 1).

Eligibility criteria included a confirmed histologic diagnosis of aggressive or indolent NHL or HD according to the REAL classification; disease stage II to IV according to the Ann Arbor staging system; Eastern Cooperative Oncology Group (ECOG) performance status less than 3; human immunodeficiency virus negativity; and normal renal, hepatic, and cardiac functions (including a baseline resting left ventricular ejection fraction (LVEF) greater than 50%). In all cases, staging evaluation included initial hematomic and chemical surveys, in addition to chest X-rays, abdominal ultrasonography, computed tomography of the chest and abdomen, and bone marrow biopsy. Other studies included lymphography (in 2 HD patients), and liver biopsy when appropriate.

No patient underwent staging laparotomy. Bulky disease was defined as a tumor mass ≥6 cm. Twenty-three patients had stage II, 15 had stage III, and 24 had stage IV disease. Twenty-two patients presented bulky disease. Thirty-eight patients had an extranodal involvement. Histologically, all 51 NHL patients had aggressive disease. Informed consent was always provided according to the Declaration of Helsinki.

Treatment protocol

The IEV schedule was as follows: ifosfamide 2,500 mg/m²/day i.v. over 4 hours followed by mesna (3 g/m²) and hydration over 10 h. daily to protect against urothelial toxicity on day 1 to 3, epirubicin 100 mg/m² i.v. on day 1; etoposide 150 mg/m² i.v. on days 1 to 3. Courses were repeated every 21 days, with a target total of three courses; chemotherapy was given in an outpatient setting. In the 45 patients for whom PBSC harvest was feasible only 2 conditioning courses were given in order to reduce tumor volume before high-dose chemotherapy and PBSC reinfusion. For these patients, subcutaneous administration of granulocyte colony-stimulating factor (G-CSF) (5 µg/kg, once daily) was commenced on day 6 and continued until completion of the PBSC harvest. The remaining 17 patients, who were considered unsuitable for myeloablative therapy, all received 3-4 cycles; G-CSF was administered at the same dose from day 6 to 14 of each cycle.

Response

All patients were restaged after completion of IEV chemotherapy. Clinical and pathologic evaluations were made by repeating radiographic investigations and bone marrow and/or liver biopsies.
whenever previous results had been positive. Complete response (CR) and partial remission (PR) were defined according to international criteria.27 No response (NR) was anything less than PR. Standard ECOG toxicity criteria were used.26

Statistical methods
Overall survival was measured from the time of entry into the protocol until death; the relapse-free interval was calculated from the date of response after autograft until relapse or death; event-free survival was measured from the autograft to the first event (resistance to treatment, relapse, death, last follow-up). Overall, relapse-free survival, and event-free survival curves were calculated according to the method of Kaplan and Meier.28 Deaths from lymphoma, secondary to lymphoma treatment or to any disease (related or unrelated) were considered an event. Analyses of prognostic factors [including the International Prognostic Factor Index, (IPI)]29 were performed with log-rank tests, Cox’s analysis,30 and logistic regression analysis.

Leukaphereses and cryoconservation
Aphereses began during recovery from myelo-suppression when the peripheral blood CD34+ cell count was >20x10^6/L. Cells were collected using a COBE cell separator. Leukapheresis was performed on consecutive days, aiming for a minimum target dose of 2x10^6/kg CD34+ cells. All PBSC products were cryopreserved with a final dimethyl sulfoxide concentration of 10% and stored at -190°C in liquid nitrogen. Patients who subsequently proceeded to autograft received conditioning therapy with either the BEAM (BCNU, etoposide, aracytin, melphalan) regimen (for HD patients) or with BAVC (BCNU, aracytin, etoposide, cyclophosphamide) regimen (for NHL patients), followed by reinfusion of the thawed PBSC product.

Results
Response
Of the 62 patients studied, 21 (34%) fulfilled the criteria for CR and 27 (44%) for PR after IEV therapy, giving an overall response rate of 78%. The remaining 14 patients were considered as having NR to the treatment. Table 2 summarizes the responses with respect to histology and status before treatment.

The overall response rates for HD and aggressive NHL were 81% and 77%, respectively. The CR rates being 45% and 32%, respectively. Among the 12 patients who were in PR before the IEV regimen, 5 (42%) achieved CR and 4 (33%) had an improved PR (a good PR; GPR). Among the patients treated with IEV after relapse, 14 (43%) achieved CR and 19 (57%) PR. Among the 19 patients with primary refractory disease, 2 (11%) achieved CR and 4 (23%) PR.

Seventeen patients were not considered available for response evaluation because of rapid disease progression (6 patients), lost to follow-up (3 patients), transplant refused (2 patients), shifting to allogeneic transplant (2 patients), or sudden disease progression with fatal outcome. Among the 17 patients who received only IEV, the 9 responders had a median duration of response of 9 months (range, 2 to 14 months).

On the other hand, 27 (60%) of the 45 patients who proceeded to autotransplantation were in CR status after grafting and 23 (51%) of them are currently still in continuous CR with a median follow-up of 25 months (range, 10-68 months). Figure 1 shows the relapse-free survival curve in patients who were in CR after the ABMT (83% at 60 months). The overall survival (Figure 2) at 60 months was 58% and event-free survival (Figure 3) was 42% at 60 months. At present, 31/62 (50%) patients are still alive and 23/62 (37%) are in continuous CR with a median follow-up of 25 months.

PBSC mobilization
Mobilization of PBSC with the IEV regimen was successful in 33/45 (74%) patients, each of whom achieved the defined minimum transplant dose of 2x10^6 CD34+ cells/kg. Of these, 19 had relapsed after front-line treatment (prior to IEV), 9 were refractory to the first-line therapy, and 5 were in PR. Among the 12 patients who failed to mobilize PBSC there were 7 pretreated (with chemotherapy regimens and mediastinal radiation therapy) refractory patients. Bone marrow was harvested from these 12 patients (7 refractory, 3 relapsed and 2 in PR). In 30/33 (91%) of the PBSC transplanted

<table>
<thead>
<tr>
<th>Disease status at time of treatment</th>
<th>No. pts</th>
<th>CR</th>
<th>PR</th>
<th>NR</th>
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<td>During PR</td>
<td>12</td>
<td>3</td>
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<td>0</td>
<td>2</td>
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<tr>
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<td>1</td>
<td>0</td>
<td>12</td>
<td>18</td>
<td>0</td>
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<td>0</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 2. Responses according to disease status prior to IEV therapy of all 62 patients.
patients, leukapheresis commenced between 13 and 15 days after starting IEV chemotherapy. The harvest of PBSC was completed after either one (n=20) or two (n=13) leukaphereses.

Toxicity
The toxicity due to IEV therapy was similar among the HD and NHL patients. The main form of toxicity was myelosuppression; grade 3-4 neutropenia was recorded following 36 (25%) of a total of 145 cycles, and grade 3-4 thrombocytopenia occurred in 25 (17%) courses. Clinically relevant infections were recorded in only 5 (8%) patients; eight (13%) required blood transfusions and 3 (5%) patients needed platelet transfusions. Although ECOG grade 3 alopecia was universal, no cardiac (monitored with LVEF), liver or renal toxicity was observed, and there were no fatalities due to side effects.

Statistical analysis
Among the ten factors (age, sex, presence/absence of bulky disease, presence/absence of B symptoms, stage, LDH level, extranodal sites, response to the front-line treatment, performance status, and IPI) investigated by Cox multivariate analysis, those most significantly associated with longer overall or relapse-free survival were good performance status (p= 0.001), the response to the front-line treatment (p= 0.001), and IPI (p= 0.026).

Discussion
The prognosis of patients with relapsed or resistant aggressive NHL and HD treated with conventional regimens remains poor. Response rates to salvage combinations generally range from 50% to 70%, with CR rates of 25 to 45%. Despite investigation of ifosfamide-etoposide or cytarabine-cisplatin-based regimens such as DHAP and ESHAP, the 2-year event-free survival rate of these patients remains at best only 10 to 15%, with less than 10% surviving for as long as 3 years. In order to increase the number of patients who could benefit from autologous bone marrow transplantation (ABMT), these forms of chemotherapy might be administered at an early time point, following an abbreviated induction therapy. There are several reports showing that ifosfamide-etoposide-containing regimens can induce responses in a wide variety of lymphoid neoplasms, both in the relapsed setting and as initial therapy, and that they can be administered with minimal toxicity. Such combinations, which use varying doses of ifosfamide, can also act as excellent stem cell mobilizers, especially when co-administered with
growth factors, thereby increasing the value of these regimens as relapse therapies.

In our study, the response rate after IEV was 78% (CR 34%, PR 44%) with no significant difference of response being found between HD patients and patients with aggressive NHL (overall response rates, 81% vs. 77%; CR rates 45% vs. 32%). Forty-five patients were studied for the feasibility of PBSC harvest, and in 33 (74%) an adequate collection was obtained. This subset of patients also included refractory cases. In addition, most of the mobilizers needed only one leukapheresis. Following the complete sequence of second-line treatment (i.e. IEV reinduction and transplantation with PBSC) 23/62 (37%) of the patients are currently in continuous CR after a median follow-up of 25 months. IEV therapy was well tolerated and there was no evidence of severe or permanent toxic effects.

Even in the refractory subset, IEV provided a relatively encouraging overall response rate (34%). Not surprisingly, the best target was the subset of relapsed patients in whom the overall response rate was 100%. The patients treated while in PR obtained an intermediate overall response rate (75%).

All the patients who received only IEV relapsed rapidly (within 14 months) after obtaining CR. In the subgroup of patients who were eligible for myeloablative treatment, we performed initial tumor debulking with IEV in order to increase the possibility of eradicating the lymphoma in the subsequent ABMT phase. Therefore, the role of subsequent ABMT with PBSC rescue seems to be pivotal in relapsed or refractory lymphoma patients. Successful mobilization of PBSC is essential for delivery of dose-intensive chemotherapy. An effective mobilization regimen forming an integral part of a planned therapeutic program should include anti-tumor activity, possibly in an outpatient setting, a planned therapeutic program should include anti-tumor activity, possibly in an outpatient setting, a

Contributions and Acknowledgments
PLZ designed the study. M.T, A.L.M., V.S, E.Z, L.A, A.G., P.A, M.S were responsible for the care of patients and data collection. F.B was responsible for the statistical analyses. All authors contributed to revising the manuscript. They are listed according to a criterion of decreasing individual contribution to the work, with the exception of the last two authors (S.T, M.B) who had a major role as senior authors in interpreting the data.

Disclosures
Conflict of interest: none.
Redundant publications: no substantial overlapping with previous papers.

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**Peer Review Outcomes**

**Manuscript processing**

This manuscript was peer-reviewed by two external referees and by Professor Gilles Salles, who acted as an Associate Editor. The final decision to accept this paper for publication was taken jointly by Prof. Salles and the Editors. Manuscript received February 5, 2002; accepted June 5, 2002.

**What is already known on this topic**

Salvage chemotherapy for relapse or refractory lymphoma patients should allow a high rate of tumor response in order to proceed with subsequent autologous stem cell transplantation. Several regimens based on the use of ifosfamide plus etoposide have been developed over the years.

**What this study adds**

This study describes the association of epirubicin with ifosfamide plus etoposide (IEV) with a high response rate and a manageable toxicity. A substantial proportion of patients received autologous stem cell transplant, three quarters of them with PBSC collected after IEV.

**Potential implications for clinical practice**

This regimen can be used as a salvage regimen for relapsing or refractory patients, allowing a good mobilization of hematopoietic stem cells.

Gilles Salles, Associate Editor