1083-8791/05/1108-0007$30.00/0
doi:10.1016/j.bbmt.2005.05.002

A New Schedule of CHOP/Rituximab Plus Granulocyte-Macrophage Colony-Stimulating Factor Is an Effective Rescue for Patients with Aggressive Lymphoma Failing Autologous Stem Cell Transplantation

Attilio Olivieri,[1] Moira Lucesole,[1] Debora Capelli,[1] Guido Gini,[1] Mauro Montanari,[1] Marco Candela,[2] Emanuela Troiani,[1] Ilaria Scortechini,[1] Antonella Poloni,[1] Pietro Leoni[1]

[1]Department of Hematology, University of Ancona, Ancona, Italy; [2]Department of Internal Medicine, University of Ancona, Ancona, Italy

Correspondence and reprint requests: Attilio Olivieri, MD, Department of Haematology, Ancona University, via Conca 1, 60100 Ancona, Italy (e-mail: a.olivieri@ao-umbertoprimo.marche.i).

Received March 8, 2005; accepted May 11, 2005

ABSTRACT

From 1999 to 2002, 20 patients with aggressive non-Hodgkin lymphoma, among 28 who failed autologous peripheral blood progenitor cell transplantation, were rescued with cyclophosphamide, hydroxydaunomycin, Oncovin (vincristine), and prednisone (CHOP)/rituximab (RTX) and granulocyte-macrophage colony-stimulating factor (GM-CSF). RTX was administered twice during each course of chemotherapy, before CHOP and after GM-CSF. This cytokine was given to increase the antibody-dependent cell-mediated cytotoxicity and to reduce the leukopenia on the basis of our preliminary data, which suggested that this cytokine can upregulate CD20 expression. The relevant (World Health Organization grade 3-4) toxicity mainly consisted of myelosuppression (neutropenia in 60% of patients). Fifteen patients achieved complete remission (CR) or had a partial response, with an overall response rate of 75% (60% CR and 15% partial response). Six of the 12 patients who achieved CR relapsed: 2 died of progressive disease, 1 died of infectious complications after allogeneic transplantation, and 3 are alive in second CR. Eight patients showed progressive disease: 5 died of progressive disease, 1 of secondary acute leukemia, and 1 of infectious complications after allogeneic transplantation, whereas 1 is alive in second CR. At last follow-up, 10 patients are alive, 6 of whom are in complete continuous remission, with a median follow-up of 31 months (range, 3-51 months). The projected 4-year progression-free survival is 31.4%, and the 4-year overall survival is 50%. This new association (RTX, CHOP, and GM-CSF) was feasible in approximately 70% of patients; the overall toxicity was manageable. The good response rate and the promising outcome observed in this subset of patients could be explained by the possible increased synergy between chemotherapy, RTX, and GM-CSF, which should be explored in further studies.

KEY WORDS

Immunotherapy • Rituximab • Lymphoma • Autologous stem cell transplantation • Failure

INTRODUCTION

Lymphoma patients who relapse after autologous peripheral blood progenitor cell transplantation (ASCT), especially those with aggressive non-Hodgkin lymphoma (NHL), have a very poor prognosis. In this setting, salvage chemotherapy or a second transplantation cannot substantially modify the very poor outcome, which is characterized by a median overall survival (OS) of 3 months for patients with large cell lymphoma [1]. Patients with mantle cell lymphoma (MCL) that relapses after ASCT also have a very poor survival [2,3].

A second transplantation attempt in these patients is rarely feasible. Previous reports suggest that a second autologous transplantation or a conventional allogeneic transplantation after the failure of autologous transplantation is rarely curative. Progressive disease and transplant-related mortality (TRM) are the primary causes of failure with second autologous and allogeneic transplantations, respectively [4].

In the allogeneic setting, the lack of suitable donors and the very high TRM reported with myeloablative conditioning [4-6] consistently reduce the proportion of patients who are candidates for this approach. However, it is very difficult to obtain a sufficient peripheral blood stem cell harvest for a second autologous bone marrow transplantation [7], and even when this procedure is feasible, the efficacy of the second attempt is often limited because of a lack of a graft-versus-tumor effect. In these patients, any salvage options are often jeopardized by both a poor performance status and a low hematologic tolerance to chemotherapy.

Rituximab (RTX) is a highly specific chimeric antibody against the CD20 antigen, which is present in most B-cell lymphomas [8,9]. RTX has demonstrated high activity both in follicular lymphoma as a single agent [10] and in aggressive NHL [11], including MCL [12,13]. In all cases, the combination of RTX with cyclophosphamide, hydroxydaunomycin, Oncovin (vincristine), and prednisone (CHOP) chemotherapy has allowed a significant increase in the response rate in follicular [14] and high grade NHL [15], even though this association has never been tested in the posttransplantation setting as a rescue therapy.

The mechanisms by which RTX induces B-cell death include antibody-dependent cell-mediated cytotoxicity (ADCC) [16], complement-mediated toxicity, and apoptosis against chemoresistant B-lymphoma cells [17]. Moreover, some observations suggest that the outcome of patients who receive RTX can be improved if the number and activity of their immune effector cells (in particular, natural killer cells) are preserved or enhanced [18].

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a cytokine that strongly increases the number and activity of polymorphonuclear cells and macrophages against opsonized targets [19-21]. Furthermore, some preliminary data suggest that this cytokine can upregulate the CD20 expression on lymphoid B cells in vitro and in vivo [22]. This study evaluated the safety and the activity of an original immunochemotherapeutic approach that included CHOP, RTX, and GM-CSF in a group of patients with aggressive NHL after ASCT failure.

MATERIALS AND METHODS

Patient Characteristics

We observed 28 patients with aggressive NHL after ASCT failure from 1999 to 2002. Twenty (14 with diffuse large cell lymphoma [DLCL] and 6 with MCL blastoid variant) were considered eligible for a salvage protocol including RTX and CHOP chemotherapy.

Exclusion criteria included a poor performance status (>1 according to the World Health Organization [WHO], not due to the underlying lymphoma), poor hematologic count (with platelets <100 000/μL and/or polymorphonuclear cells <1500/μL), leukemic disease with hyperleukocytosis (white blood cell count >50 000/μL), HLA-compatible donor if aged <55 years, previous anthracycline cumulative dose >300 mg/m^2, cardiac ejection fraction <45%, central nervous system involvement, positive serologic test findings for human immunodeficiency virus, and active hepatitis B. Eight (29%) of the 28 observed patients were not eligible for the following reasons: 3 had previously received an anthracycline cumulative dose >300 mg/m^2, 3 had a very poor performance status unrelated to the underlying lymphoma, 1 had a poor hematologic count, and 1 refused the treatment. Patient characteristics are shown in Table 1.

Patients were heavily pretreated with a median number of 3 chemotherapy regimens before ASCT: 9 patients with DLCL had previously been enrolled in a protocol of high-dose sequential therapy including the etoposide, doxorubicin, cyclophosphamide, vincristine, prednisone, and bleomycin (VACOP-B) regimen (a median of 8 weeks with a median cumulative dose of 200 mg/m^2 doxorubicin), followed by cyclophospha-

Table 1. *Patient Characteristics*

Variable	No. of Patients	%
Age (y)		
Median	48	
Range	28-69	
Sex		
Male	6	30
Female	14	70
Status		
PR	2	10
Relapse	16	80
Progression	2	10
Previous CHT regimens		
Median	4	
Range	2-7	
Bulky disease (after transplantation)		
Yes	7	35
No	13	65
Bone marrow involvement		
Yes	8	40
No	12	60
WHO performance status		
0-2	17	85
3-4	3	15
Histologic type		
Diffuse large cell lymphoma	14	70
Mantle cell lymphoma (blastoid variant)	6	30
Stage		
I-II	2	10
III-IV	18	90
IPI score		
0-1	1	5
2	6	30
3	9	45
4-5	4	20

CHT indicates chemotherapy; IPI, International Prognostic Index.

Table 2. *Toxicity*

Variable	176 RTX Infusions 73 CHOP + RTX and 10 Second Step Immunotherapy (% Patients with an Event in at Least 1 Cycle)	
	PS 1-2 (WHO)	PS 3-4 (WHO)
Hematologic toxicity		
Anemia	15	40
Neutropenia	5	60
Thrombocytopenia	5	50
Extrahematologic toxicity		
Infection	10	15
Mucositis	10	0
Liver toxicity	5	5
Cardiac toxicity	0	0
Neurologic toxicity	25	0
Renal toxicity	10	0
Lung toxicity	5	0
Nausea and vomiting	5	0
Constipation	10	5

PS indicates performance status.

mide 7 g/m^2 and VP-16 2 g/m^2 before ASCT. The other 5 patients with DLCL received ASCT for relapse after 12 VACOP-B courses (with a median cumulative dose of 300 mg/m^2 doxorubicin) and received 2 or 3 dexamethasone, high-dose cytarabine, and Platinol (cisplatin) (DHAP) courses before high-dose therapy; the 6 patients with MCL received ASCT as second-line salvage therapy after 2 or 3 courses of DHAP.

Before enrollment, all patients were required to give their written informed consent; to have confirmation of active CD20$^+$ NHL by biopsy or fine-needle aspiration of an involved site (all biopsy specimens were reviewed by 1 or 2 hematopathologists); to be younger than 70 years of age; to show a relapse, progression, or persistence of disease after ASCT; and to have measurable disease and absence of severe organ dysfunctions (bilirubin <3 mg/dL, creatinine <2 mg/m, alanine aminotransferase and aspartate aminotransferase <3 times the normal values, and carbon monoxide diffusion in the lung >40%) not related to the underlying disease. All patients underwent pretreatment staging studies that included computed tomography of the chest, abdomen, and pelvis; nuclear imaging with gallium scans, fluorine-18-fluorodeoxyglucose positron emission tomography, or both; and unilateral bone marrow biopsies. Our institutional review board approved this study.

Treatment

Patients received a scheme of treatment that consisted of a modified CHOP-21 schedule (cyclophosphamide 750 mg/m^2, doxorubicin 50 mg/m^2, and vincristine 1.4 mg/m^2 up to a maximal dose of 2 mg on day 4, and prednisone 40 mg/m^2/d for 4 days, from day 4 to day 7). Patients also received RTX 375 mg/m^2 on day 1 and 375 mg/m^2 on day 14 of each cycle of CHOP. The RTX infusion was interrupted in the event of fever, chills, edema, congestion of the head and neck mucosa, hypotension, or any other serious adverse event and was resumed when such event was no longer observed. GM-CSF was administered subcutaneously at 150 μg/d; it was started the day after the end of CHOP (day 5) and was continued for at least 9 days until the second RTX administration (Figure 1). The rationale for GM-CSF administration on the fourth day was based on the well-documented ability of this drug to prevent or reduce neutropenia after chemotherapy, on data supporting increased ADCC by phagocytic cells after GM-CSF administration [20,21], and, finally, on the possibility of upregulating CD20 expression, as suggested also by our preliminary findings [22,23] (Figure 2). Patients were treated every 3 weeks for at least 2 courses before the first evaluation of response.

If the absolute neutrophil count was $<1.5 \times 10^9$/L or the platelet count was $<1 \times 10^9$/L, chemotherapy administration was delayed for up to 2 weeks, and then

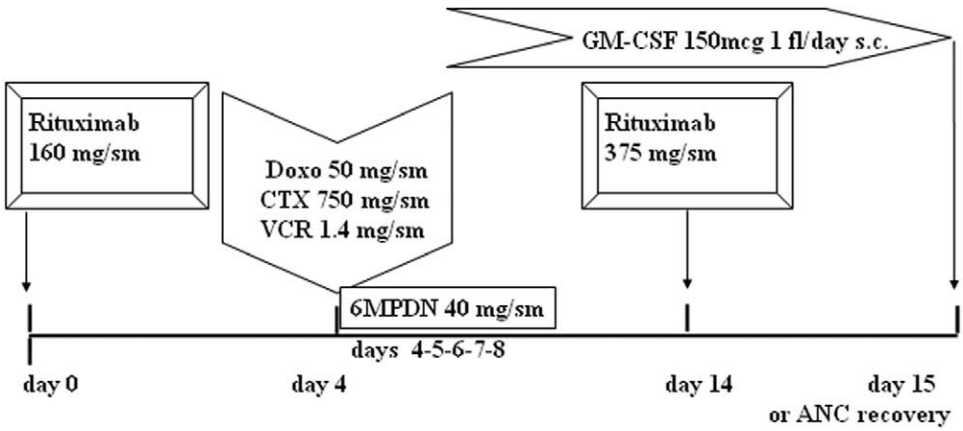

Figure 1. Schedule of treatment in 20 patients with aggressive lymphoma and failed ASCT. Doxo indicates doxorubicin; CTX, cyclophosphamide; VCR, vincristine; ANC, absolute neutrophil count; sm, square meter; MPDN, prednisone.

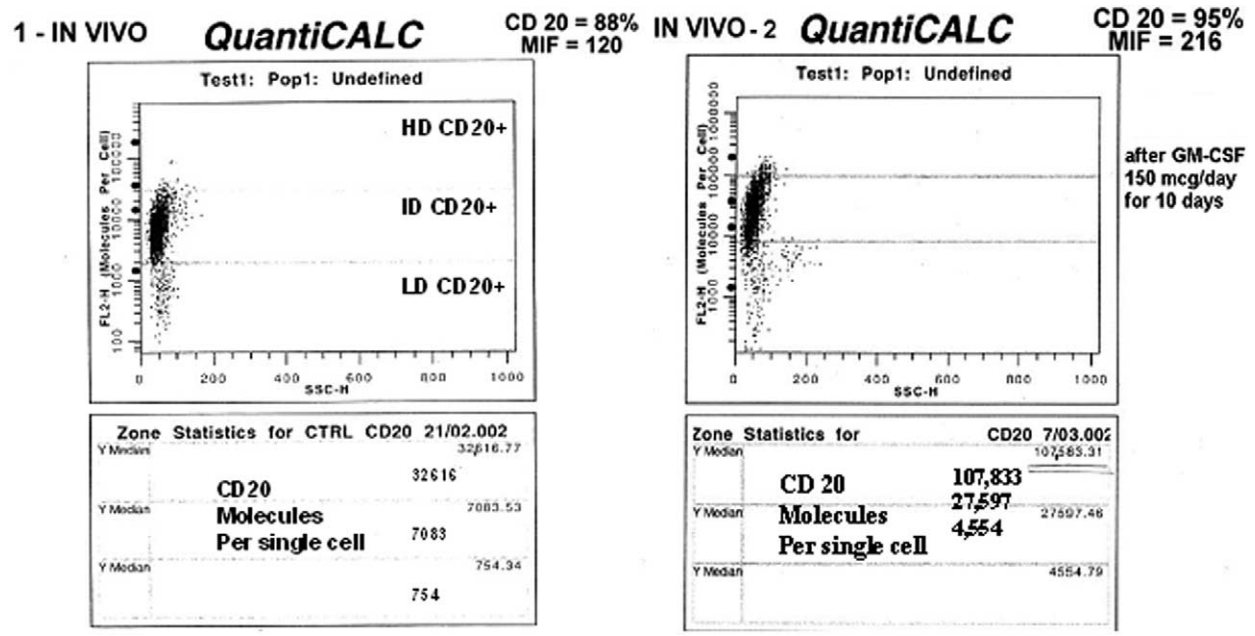

Figure 2. Effects of GM-CSF administration for 10 days in a woman with leukemic MCL; $CD20^+$ cells increased from 80% to 95%, and median-intensity fluorescence (MIF) increased from 120 to 216. The QuantiCALC assay allowed enumeration of the CD20 density per single cell, which increased from 32 616 to 107 833 in the high density cluster (upper), from 7 083 to 27 597 in the intermediate density cluster (medium), and from 755 to 455 in the low density cluster (down) respectively, in the lymphoma cells.

treatment was stopped. The doses of RTX were not modified, and RTX was also continued when CHOP was stopped. Treatment was stopped if lymphoma progressed, if the patient declined to continue, or at the discretion of the investigator in cases of intercurrent illness or adverse events.

Patients showing disease progression after 2 courses of treatment were excluded from further treatment. The responders (complete remission [CR] or partial response [PR]) received 2 more courses of CHOP/RTX/GM-CSF or 3 more courses of RTX alone in case of poor hematologic tolerance, at the dose of 375 mg/m^2 at day 7, every 21 days, together with daily GM-CSF from day 1 to day 11 (second step of therapy; Figure 3).

Patients achieving clinicoradiologic CR did not receive any further treatment. Patients in PR after the second step of therapy were treated at the clinician's discretion (local radiotherapy or allogeneic transplantation in case of HLA-matched sibling availability).

Response to Treatment and Adverse Events

The first tumor response assessment was performed after at least 2 courses of chemotherapy (including 4 doses of RTX), and the final restaging was performed at the end of treatment. The responses were classified as CR, PR, or progressive disease according to the International Workshop criteria [24].

CR was defined as the disappearance of all lesions and radiologic or biological abnormalities observed at diagnosis and the absence of new lesions. An unconfirmed CR was defined as a CR with the persistence of some radiologic abnormalities, which had to be regressed in size by at least 75%. PR was defined as the regression of all measurable lesions by more than 50%, the disappearance of no measurable lesions, and the absence of new lesions. Progressive disease was defined as the appearance of a new lesion, any growth of the initial lesion by more than 25%, or growth of any measurable lesion that had regressed during treatment by more than 50% from its smallest dimensions.

All adverse events reported by the patient or observed by the investigator were collected from the case-report form in predefined categories. An adverse event was defined as any adverse change from the patient's baseline condition, whether it was considered related to treatment or not. Each event was graded according to the WHO toxicity criteria.

Statistical Analysis

Because of the scarcity of data in patients with aggressive lymphomas treated after ASCT failure, the safety and efficacy evaluation of this schedule was powered by basing our statistical design on the historical experience reported in 54 patients with refractory or relapsing aggressive lymphoma (DLCL or MCL) rescued with RTX alone (375 or 500 mg/m^2). Coiffier et al. [11] reported an overall response rate (ORR) of 32%, with a CR rate of 9% and a treatment-related death rate of 4%.

With a probability of early termination of 55%, the following rules were established for the prelimi-

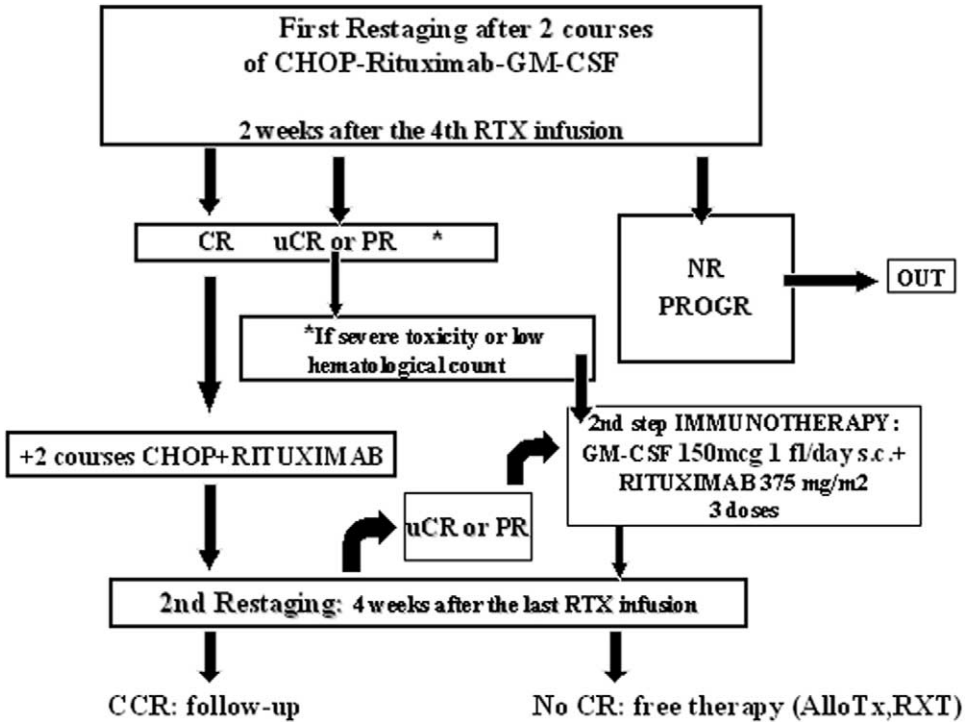

Figure 3. First restaging after 2 courses of CHOP/rituximab/GM-CSF, 2 weeks after the fourth RTX infusion. AlloTx indicates allogeneic transplantation; uCR, CR unconfirmed.

nary evaluation of safety and efficacy in the first 15 patients: with an observed rate of CR <2 in 15 or with a treatment-related death rate >1 in 15, the study should be stopped. We hypothesized that our schedule could obtain a CR rate >29% (>20% than the historical data); therefore, the expected sample size (considering $\alpha = .05$ and $\beta = .20$) was 20 patients with a minimax Simon design [25].

OS and progression-free survival (PFS) were calculated from the day the CHOP/RTX/GM-CSF treatment started until death due to any cause or last follow-up. The probabilities of survival and relapse were estimated and plotted by using the Kaplan-Meier method. Data were analyzed by using the SPSS statistical package (SPSS Inc., Chicago, IL).

RESULTS

The median time from transplantation to the initiation of treatment in the 20 patients was 8 months (range, 2-72 months). The 4 scheduled courses of CHOP/RTX/GM-CSF were given to 15 patients (75% of enrolled patients), and 5 patients showed disease progression after the first or second cycle. These patients were included in the response and outcome analysis according to intention-to-treat criteria. Two patients who achieved a PR after 4 courses of CHOP/RTX/GM-CSF received 2 additional courses, followed by the second step of therapy. One patient in PR after the 4 courses of chemoimmunotherapy withdrew from the protocol because of persistent aplasia and eventually had progressive disease; among the 12 patients in CR, 8 patients with unconfirmed CR after the 4 courses of CHOP/RTX/GM-CSF received the second step of therapy as previously planned.

Toxicity

All 20 patients were included for the toxicity evaluation of 73 courses of CHOP/RTX/GM-CSF, followed in 10 patients by the second step of immunotherapy, for a total of 176 RTX infusions. The GM-CSF administration was interrupted in 3 patients because of intolerance and was replaced by granulocyte colony-stimulating factor. These 3 patients developed an

Table 3. *Response to Therapy in the 20 Patients with Aggressive NHL and in the Subgroup of the 14 Patients with DLCL*

Response	NHL, n (%)	DLCL NHL, n (%)
Early death	0/20 (0)	0/14 (0)
CR	12/20 (60)	9/14 (64)
PR	3/20 (15)	1/14 (7)
PD/NR	5/20 (25)	4/14 (29)
ORR	15/20 (75)	10/14 (71)
Follow-up		
Early death	0/20 (0)	0/14 (0)
CCR	6/20 (30)	6/14 (43)
PD	8/20 (40)	5/14 (36)
Relapse	6/20 (30)	3/14 (21)

PD indicates progressive disease; NR, no response.

Table 4. *Clinical Outcome of the 20 Patients According to the Initial Characteristics and the Secondary Therapy Given after CHOP/RTX/GM-CSF Failure*

Patient No.	Age (y)	Histology	IPI	Time ASCT Prior CHOP-RTX (mo)	Response CHOP-RTX	Outcome	Secondary Therapy	Status at Last Follow-Up	Follow-Up (mo)
1	65	MCL	3	15	CR	Relapse	Allotransplantation	Death	23
2	63	DLCL	3	12	CR	Relapse	No therapy	Death	30
3	67	MCL	5	5	CR	Relapse	No therapy	Death	36
4	69	MCL	2	72	CR	Relapse	LPD + RTX	CR	49*
5	28	DLCL	2	5	CR	CCR	No therapy	CR	51*
6	36	DLCL	3	2	CR	Relapse	Allotransplantation	CR	48*
7	36	DLCL	3	44	NR	Progression	No therapy	Death	4
8	36	DLCL	4	2	NR	Progression	No therapy	Death	3
9	58	DLCL	2	35	CR	Relapse	LPD + RTX	CR	38*
10	43	DLCL	4	20	NR	Progression	Allotransplantation	Death	9
11	37	DLCL	3	7	CR	CCR	No therapy	CR	45*
12	48	DLCL	3	7	CR	CCR	No therapy	CR	45*
13	51	DLCL	2	7	CR	CCR	No therapy	CR	45*
14	66	MCL	3	8	PR	Progression	No therapy	Death	24
15	49	MCL	4	3	NR	Progression	No therapy	Death	3
16	33	DLCL	1	5	NR	Progression	No therapy	Death	14
17	40	MCL	3	29	PR	Progression	Allotransplantation	CR	35*
18	43	DLCL	3	70	PR	Progression	No therapy	Death	6
19	66	DLCL	2	33	CR	CCR	No therapy	CR	32*
20	48	DLCL	2	8	CR	CCR	No therapy	CR	31*

IPI indicates International Prognostic Index; F.U., follow-up; NR, no response; LPD, liposomal pegylated doxorubicin.
*Alive and well.

acute syndrome after the first GM-CSF dose, with chills and fever in 1 case and severe bone pain and chest pain (without evidence of coronary disease) in the other 2 patients. RTX was generally well tolerated. We observed 1 grade 4 tumor lysis syndrome and 2 grade 1 to 2 episodes of hypotension after the first infusion of RTX. The grade 3 to 4 adverse effects were consistent with the expected toxicity of CHOP chemotherapy. We did not observe any toxic deaths. The most relevant (WHO 3-4) toxicity was hematologic: 1 patient, with a long disease history and heavy pretreatment, withdrew from the protocol because of persistent marrow aplasia.

Sixty percent of patients developed severe neutropenia, and 50% and 40% of patients experienced severe anemia and thrombocytopenia, respectively. The grade 3 to 4 extrahematologic toxicity mainly consisted of infectious complications (15%): we observed 1 *Pseudomonas aeruginosa* sepsis, 1 *P. aeruginosa* pneumonitis, and 1 radiologically documented pneumonitis. Grade 1/2 and grade 3/4 hematologic and extrahematologic toxicities are reported in Table 2.

Response and Survival Analysis

Fifteen patients responded to treatment, with 12 (60%) CRs and 3 PRs (ORR, 75%), whereas 5 patients were classified as nonresponders. Six patients are still in complete continuous remission (CCR) with a median follow-up of 31 months (range, 3-51 months).

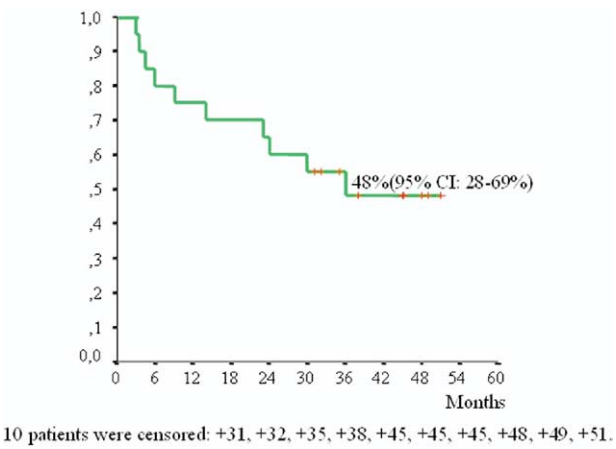

Figure 4. OS in 20 patients with aggressive NHL. CI indicates confidence interval.

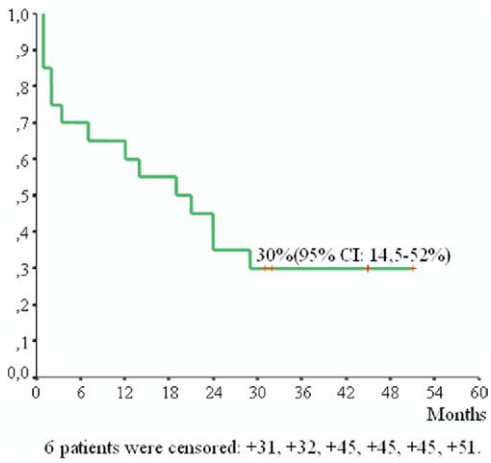

Figure 5. PFS in 20 patients with aggressive NHL. CI indicates confidence interval.

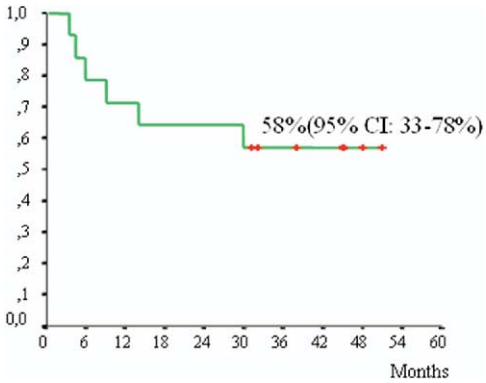

Figure 6. OS in 14 DLCL patients. CI indicates confidence interval.

Eight patients experienced disease progression: 5 died of progressive disease, 1 died of infectious complications after receiving an allogeneic transplant, 1 died of secondary acute myeloid leukemia, and 1 is alive and in second CR after receiving an allogeneic transplant from an HLA-identical sibling.

Six of the 12 patients who achieved CR relapsed: 2 died of progressive disease, 1 died of infectious complications after allogeneic transplantation, and 3 are alive and achieved a second CR (2 after a liposomal anthracycline-based regimen and 1 after an allogeneic transplantation). The ORR was 71% in the 14 patients with DLCL and 83% in the 8 MCL patients, with a 64% and 50% CR rate, respectively. Nevertheless, despite this apparently similar response rate, all 5 MCL patients who were responsive to the chemotherapy eventually relapsed or progressed within a median time of 14 months, compared with the 3 relapses observed in the 9 DLCL patients who achieved CR.

All 20 patients were evaluable for clinical outcome with a median follow-up of 31 months (range, 3-51 months). Ten patients died; 7 of these died of progressive disease (6 with DLCL NHL and 1 with MCL). As far as the other 3 patients affected by MCL are concerned, 1 died of pneumonia, 1 died of sepsis after allogeneic transplantation performed after the relapse, and 1 died of secondary acute leukemia.

At the last follow-up, 10 patients were alive, among whom 2 were affected by MCL and 8 by DLCL. Six patients were still in CCR, and 4 achieved a subsequent CR: 2 after allogeneic transplantation and 2 after RTX/liposomal anthracycline–based regimens. These data are summarized in Tables 3 and 4.

The projected 4-year OS was 48% (95% confidence interval, 28%-69%; Figure 4), and the 4-year PFS was 30% (95% confidence interval, 14.5%-52%; Figure 5). In the 14 patients with DLCL, the OS was 58%, with 43% PFS (Figures 6 and 7).

DISCUSSION

The first attempt at rescue therapy with RTX alone achieved only a 31% ORR in pretreated aggressive NHL [11]. After the demonstration that the RTX association with CHOP was very effective as up-front therapy both in patients with indolent NHL [14] and in those with aggressive NHL [11,26], many other attempts to improve the response rate have been tested that associate RTX with other chemotherapy regimens [27].

We decided to modify the timing of RTX administration during CHOP therapy, basing our schedule on the hypothesis that RTX can sensitize lymphoma cells to chemotherapy [17] and that GM-CSF administration can increase RTX activity, both by upregulating CD20 expression [19,20] and by enhancing ADCC [21-23]. Moreover, in vivo and in vitro experiments suggest that low doses of GM-CSF can stimulate and expand both the natural killer compartment and ADCC [19-21]. Many patients showed a poor hematologic tolerance to chemotherapy after ASCT, and profound immune suppression was documented in all of them even 1 or 2 years after ASCT [28].

In patients with a suitable donor, a second allogeneic transplantation can offer a better chance of cure than a second ASCT, thanks to the potential graft-versus-leukemia effect, but this procedure is affected by a very high TRM [29]. Recently, the use of reduced-intensity conditioning regimens has reduced TRM with a very promising OS and PFS, even in heavily pretreated patients with hematologic malignancies [30]. The role of reduced-intensity conditioning after ASCT failure has been recently investigated in 38 patients with lymphoproliferative malignancies [31]. This approach was feasible, with a 20% TRM at 14 months, 53% OS, and 50% PFS; nevertheless, only a minority of patients, after ASCT failure, could benefit from this approach.

The role of RTX as salvage therapy after ASCT failure has not been extensively investigated in pa-

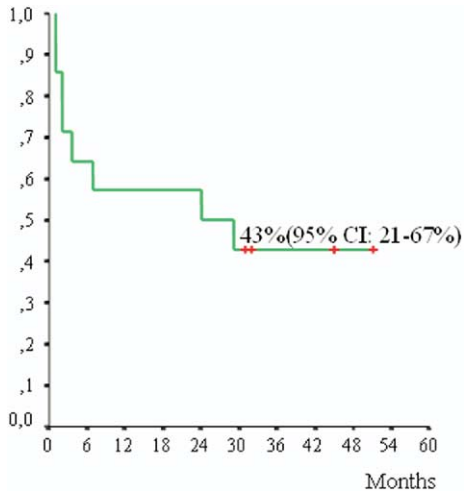

Figure 7. PFS in 14 DLCL patients. CI indicates confidence interval.

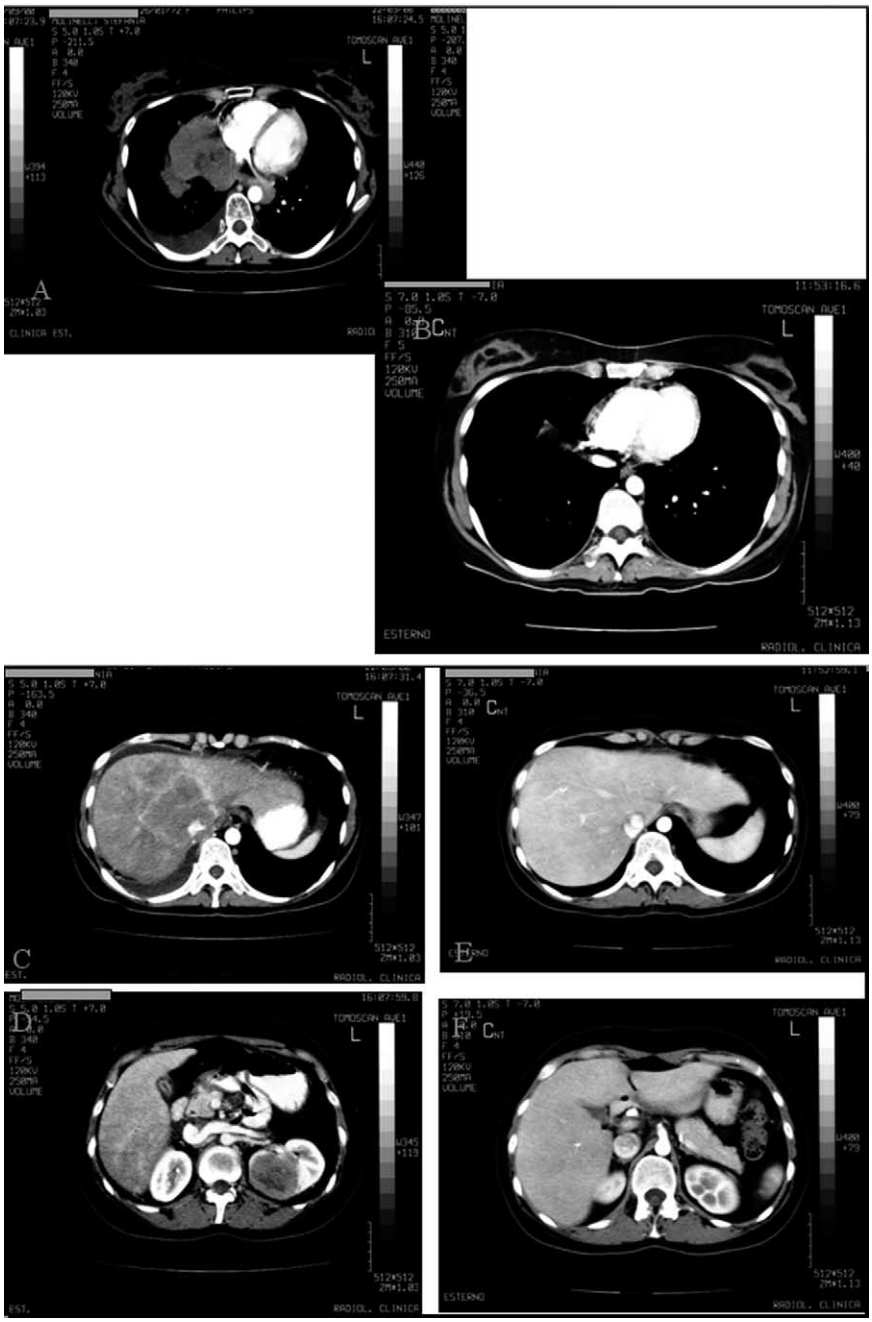

Figure 8. Computed tomographic scan of a young woman after a DLCL relapse 6 months after ASCT, with mediastinal bulky disease involving the lung and pleural effusion (A) after 4 courses of CHOP/GM-CSF/RTX (B). The same patient (C and D) showed extensive liver and renal involvement at relapse. E and F, Complete regression of lymphoma. The patient is in CCR 4 years after the end of therapy.

tients with $CD20^+$ DLCL [32]; recently, 17 patients received RTX alone after ASCT failure, with a 54% ORR, but the median duration of response was only 13 months [33]. More recently, 28 lymphoma patients received RTX after ASCT (n = 16) or allogeneic stem cell transplantation (n = 12); 9 patients who did not achieve CR after stem cell transplantation converted to CR after RTX [34].

In a second report, a heterogeneous population including 55 patients with NHL and Hodgkin disease received RTX plus GM-CSF after ASCT. Among the 33 patients with B-cell lymphoma, 14 had primary refractory disease, 12 had relapsed disease, and 7 had high-risk disease in first CR [35].

A third report evaluated the efficacy and toxicity of RTX plus GM-CSF after ASCT in 35 patients with DLCL (n = 25), MCL (n = 3), or other subtypes of B-cell lymphoma. With a median follow-up of 30 months, the 2-year event-free survival rate was 83%, and OS was 88%. The third report, although it confirms the feasibility of the association RTX plus GM-CSF in the posttransplantation

setting, does not answer the question of whether this association could be worthwhile in patients whose ASCT fails; indeed, 34 of 35 patients had chemosensitive disease before ASCT, and an amendment of the protocol also allowed enrollment of patients in CR before ASCT [36].

Our approach, tested in a homogeneous setting, was feasible in most (20 of 28) patients with aggressive lymphoma whose ASCT failed. We did not observe severe or fatal cardiotoxicities, probably because we included only patients with a relatively low cumulative dose (<300 mg/m^2) of doxorubicin.

In the 14 patients with DLCL, the outcome seems to be better than reported in previous experiences, thus suggesting that this schedule could effectively eradicate the disease in a relevant proportion (6/14) of patients. Indeed, we observed a long CCR in patients with DLCL relapsing with bulky disease and extra nodal sites after high dose sequence followed by ASCT (Figure 8). Conversely, although they showed a very high ORR, none of the 6 MCL patients maintained a CCR.

In conclusion, our study shows that this new schedule represents an effective salvage treatment for lymphoma patients with failed ASCT, who are often not eligible for allogeneic transplantation. More data are needed to confirm the synergy between GM-CSF and RTX, but in our opinion, this association should be now considered for all patients with CD20$^+$ DLCL and failed ASCT who lack a suitable donor or who are not eligible for an allogeneic bone marrow transplantation.

In many centers, the anthracycline-based regimens plus RTX are increasingly used as up-front treatment for young patients with CD20$^+$ DLCL, and it is not uncommon to observe many patients who have received a heavy cumulative anthracycline dose after their first- or second-line therapy fails. In this case, although the re-treatment with RTX can be safe and effective in patients with bulky disease [37], if the previous cumulative dose of doxorubicin is >400 mg/m^2, rescue therapy with CHOP/RTX plus GM-CSF could generate some concerns about the high risk of cardiotoxicity or acquired drug resistance. For this reason, we think that the introduction of liposomal anthracyclines (instead of conventional doxorubicin) in the same schedule could be an attractive alternative option for this subset of patients.

ACKNOWLEDGMENTS

This work was supported by Associazione Italiana per la Lotta Contro le Leucemie, Linfomi e Mielomi Sezione di Ancona.

REFERENCES

1. Vose JM, Bierman PJ, Anderson JR, et al. Progressive disease after high-dose therapy and autologous transplantation for lymphoid malignancy: clinical course and patient follow-up. *Blood.* 1992;15:2142-2148.
2. Weaver CH, Appelbaum F, Petersen FB, Buckner CD. Follow-up report on the outcome of patients relapsing after autologous marrow transplantation for malignant lymphoma. *J Clin Oncol.* 1993;11:812-813.
3. Freedman AS, Neuberg D, Gribben JG, et al. High-dose chemoradiotherapy and anti B cell monoclonal antibody purged autologous bone marrow transplantation in mantle-cell lymphoma: no evidence for long time remission. *J Clin Oncol.* 1998;16:13-18.
4. De Lima M, van Besien KW, Giralt SA, et al. Bone marrow transplantation after failure of autologous transplant for non Hodgkin's lymphoma. *Bone Marrow Transplant.* 1997;19:121-127.
5. Tsai T, Goodman S, Saez R, et al. Allogenic bone marrow transplantation in patients who relapse after autologous transplantation. *Bone Marrow Transplant.* 1997;20:859-863.
6. Radich JP, Gooley T, Sanders JE, Anasetti C, Chauncey T, Appelbaum FR. Second allogenic transplantation after failure of first autologous transplantation. *Biol Blood Marrow Transplant.* 2000;6:272-279.
7. Haas R, Mohle R, Fruhauf S, et al. Patient characteristics associated with successful mobilizing and autografting of peripheral blood progenitor cells in malignant lymphoma. *Blood.* 1994;83:3787-3794.
8. Reff ME, Carner K, Chambers KS, et al. Depletion of B cells in vivo by a chimeric mouse human monoclonal antibody to CD20. *Blood.* 1994;83:435-445.
9. Grillo Lopez AJ. Rituximab: an insider's historical prospective. *Semin Oncol.* 2000;27(suppl 12):9-16.
10. McLaughlin P, Hagemeister FB, Grillo-Lopez AJ. Rituximab in indolent lymphoma: the single-agent pivotal trial. *Semin Oncol.* 1999;26(suppl 14):79-87.
11. Coiffier B, Haioun C, Ketterer N, et al. Rituximab (anti-CD20 monoclonal antibody) for the treatment of patients with relapsing or refractory aggressive lymphoma: a multicenter phase II study. *Blood.* 1998;92:1927-1932.
12. Howard OM, Gribben JG, Neuberg DS, et al. Rituximab and CHOP induction therapy for newly diagnosed mantle-cell lymphoma: molecular responses are not predictive of progression free survival. *J Clin Oncol.* 2002;20:1288-1294.
13. Gianni AM, Cortelazzo S, Magni M, Martelli M. Rituximab: enhancing stem cell transplantation in mantle cell lymphoma. *Bone Marrow Transplant.* 2002;29(suppl 1):S10-S13.
14. Czuczman MS, Grillo-Lopez AJ, White CA, et al. Treatment of patients with low grade B-cell lymphoma with the combination of chimeric anti-CD20 monoclonal antibody and CHOP chemotherapy. *J Clin Oncol.* 1999;17:268-276.
15. Coiffier B, Lapage E, Briere J, et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large cell lymphoma. *N Engl J Med.* 2002;346:235-242.
16. Flieger D, Renoth S, Beier I, Sauerbruch T, Schmidt-Wolf I. IDEC-C2B8 in CD20-expressing lymphoma cell lines. Mechanism of cytotoxicity induced by chimeric mouse human monoclonal antibody. *Cell Immunol.* 2000;204:55-63.
17. Wilson W. Chemotherapy sensitization by rituximab: experimental and clinical evidence. *Semin Oncol.* 2000;27(6 suppl 12):30-36.

18. Wilson S, Hurst D, Yuen A, Gluck L. IL-2-mediated NK cell expansion correlates with clinical response to rituximab: results of 2 phase I trials of the combination of IL-2 and rituximab. *Blood*. 2001;98:2525a:602.
19. Bober LA, Grace MJ, Pugliese-Sivo C, Rojas-Triana A, Sullivan LM, Narula SK. The effect of GM-CSF and G-CSF on human neutrophil function. *Immunopharmacology*. 1995;29:111-119.
20. Nagler A, Shur I, Barak V, Fabian I. Granulocyte-macrophage colony-stimulating factor dependent monocyte-mediated cytotoxicity post-autologous bone marrow transplantation. *Leuk Res.* 1996;20:637-643.
21. Stockmeyer B, Elsasser D, Dechant M, et al. Mechanisms of G-CSF- or GM-CSF-stimulated tumor cell killing by Fc receptor-directed bispecific antibodies. *J Immunol Methods*. 2001; 248:103-111.
22. Venugopal P, Sivaraman S, Huang X, et al. Upregulation of CD20 antigen expression in chronic lymphocytic leukemia (CLL) cells by in vitro exposure to cytokines. *Blood*. 1998; 92(suppl 1):1009.
23. Olivieri A, Lucesole M, Capelli D, et al. GM-CSF addition to rituximab improves the response rate and survival in patients with non-Hodgkin's lymphoma refractory or relapsed after bone marrow transplantation. *Haematol*. 2001;86(suppl 10):31.
24. Cheson BD, Horning SJ, Coiffier B, et al. Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. NCI Sponsored International Working Group. *J Clin Oncol.* 1999;17:1244.
25. Simon R. Optimal two stage designs for phase II clinical trials. *Control Clin Trials.* 1989;10:1-10.
26. Vose JM, Link BK, Grossbard ML, et al. Phase II study of rituximab in combination with CHOP chemotherapy in patients with previously untreated, aggressive non-Hodgkin's lymphoma. *J Clin Oncol.* 2001;19:389-397.
27. Kewalramani T, Zelenetz AD, Nimer SD, et al. Rituximab and ICE as second-line therapy before autologous stem cell transplantation for relapsed or primary refractory diffuse large B-cell lymphoma. *Blood.* 2004;103:3684-3688.
28. Lum LG. The kinetics of immune reconstitution after human marrow transplantation. *Blood.* 1987;69:369-380.
29. Van Besien K, Sobocinski KA, Rowlings PA, Murphy SC, Armitage JO, Bishop MR. Allogenic bone marrow transplantation for low-grade lymphoma. *Blood.* 1998;92:1832-1836.
30. Corradini P, Tarella C, Olivieri A, et al. Reduced intensity conditioning followed by allografting of hematopoietic cells can produce clinical and molecular remissions in patients with poor-risk hematologic malignancies. *Blood.* 2002;99:75-82.
31. Branson K, Chopra R, Kottaridis PD, et al. Role of nonmyeloablative allogeneic stem-cell transplantation after failure of autologous transplantation in patients with lymphoproliferative malignancies. *J Clin Oncol.* 2002;20:4022-4031.
32. Kewalramani T, Nimer SD, Zelenetz AD, et al. Progressive disease following autologous transplantation in patients with chemosensitive relapsed or primary refractory Hodgkin's disease or aggressive non Hodgkin's lymphoma. *Bone Marrow Transplant.* 2003;32:673-679.
33. Tsai DE, Moore HCF, Hardy CL, et al. Rituximab (anti-CD20 monoclonal antibody) therapy for progressive intermediate-grade non-Hodgkin's lymphoma after high-dose therapy and autologous peripheral stem cell transplantation. *Bone Marrow Transplant.* 1999;24:521-526.
34. Shimoni A, Hardan I, Avigdor A, et al. Rituximab reduces relapse risk after allogeneic and autologous stem cell transplantation in patients with high risk aggressive non Hodgkin's lymphoma. *Br J Haematol.* 2003;122:457-464.
35. Rapoport AP, Meisenberg B, Sarkodee-Adoo C, et al. Autotransplantation for advanced lymphoma and Hodgkin's disease followed by post-transplant rituxan/GM-CSF or radiotherapy and consolidation chemotherapy. *Bone Marrow Transplant.* 2002;29:303-312.
36. Horwitz SM, Negrin RS, Blume KG, et al. Rituximab as adjuvant to high-dose therapy and autologous hematopoietic cell transplantation for aggressive non-Hodgkin lymphoma. *Blood.* 2004;103:777-783.
37. Davies TA, Grillo Lopez AJ, White CA, et al. Final report on the safety and efficacy of retreatment with Rituximab for patients with non Hodgkin's lymphoma [abstract]. *Blood*. 1999; 94(10):88a.