Dose-dense and high-dose chemotherapy plus Rituximab with autologous stem cell transplantation for primary treatment of diffuse large B-cell lymphoma at poor prognosis: a phase II multicenter study

by Umberto Vitolo, Annalisa Chiappella, Emanuele Angelucci, Giuseppe Rossi, Anna Marina Liberati, Maria Giuseppina Cabras, Barbara Botto, Giovanni Niccone, Gianluca Gaidano, Lorenzo Falchi, Roberto Freilone, Domenico Novero, Lorella Orsucci, Vincenzo Pavone, Enrico Pogliani, Delia Rota-Scalabrin, Flavia Salvi, Anna Tonso, Alessandra Tucci, and Alessandro Levis on behalf of Gruppo Italiano Multiregionale Linfomi e Leucemie (GIMURELL)

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ABSTRACT

Background
We investigated the addition of Rituximab to dose-dense and high-dose chemotherapy (HDC) with autologous stem-cell transplantation (ASCT) in untreated poor-prognosis diffuse large B-cell lymphoma (DLBCL).

Design and Methods
Ninety-four young (age 18-60), stage III-IV patients at age-adjusted International Prognostic Index (aa-IPI) intermediate/high or high risk were enrolled into a phase II trial. Treatment was given as follows: four courses of bi-weekly R-MegaCEOP14 (Rituximab-cyclophosphamide-epirubicine-vincristine-prednisone), two courses of R-MAD (Rituximab-mitoxantrone-cytarabine-dexamethasone) and BEAM (carmustine-etoposide-cytarabine-melphalan) with ASCT.

Results
Complete responses and toxic deaths were: 82% and 5% respectively. Four-year failure-free survival (FFS) and four-year overall survival (OS) rates were: 73% and 80% respectively. These patients (R-HDC group) were retrospectively compared to 41 patients with similar characteristics enrolled into a previous phase II trial of HDC without Rituximab treated with: eight weekly infusions of MACOP-B (methotrexate-doxorubicin-cyclophosphamide-vincristine-prednisone-bleomycin), two MAD courses and BEAM with ASCT (HDC historic control group). The outcome for R-HDC compared to HDC group was: four-year FFS 73% versus 44% (p=0.001) and four-year OS 80% versus 54% (p=0.002), respectively. A Cox’s multivariable model was performed to adjust the effect of treatment for unbalanced or important prognostic factors: failure and death risks were significantly reduced in R-HDC group compared to HDC group, with an adjusted hazard ratio of 0.44 (p=0.01) for FFS and 0.46 (p=0.02) for OS.

Conclusions
These results suggest that the addition of Rituximab to HDC is effective and safe in DLBCL with a poor-prognosis and they need to be compared to dose-dense chemiomunotherapy without ASCT into randomized trials. Registered at http://www.clinicaltrials.gov: NCT00556127.

Key words: high-dose chemotherapy, Rituximab, large B-cell lymphoma, multicenter study.


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Introduction

Patients with diffuse large B-cell lymphoma (DLBCL) with an intermediate/high or high-risk according to the age-adjusted International Prognostic Index (aa-IPI) have a dismal prognosis with responsive five-year survival rates of 46% and 32%. High-dose chemotherapy (HDC) with autologous stem cell transplantation (ASCT) showed an effective salvage treatment for chemo-sensitive relapsed patients. These results prompted many investigators to apply this approach as part of the initial therapy for patients with DLBCL, especially for those with a poor prognosis. So far, no clear benefits were shown in such patients, and conflicting results were generated in randomized studies, with similar survival rates in patients receiving either first-line HDC and ASCT, or standard chemotherapy without Rituximab.5-11

The addition of Rituximab to CHOEP21 or dose-dense CHOEP14 significantly improves the overall and event-free survival compared with CHOEP alone either in elderly or in young low-risk patients with DLBCL.12-15 However, less data are available in young DLBCL patients with a poor prognosis.16 The combination of Rituximab with dose-dense chemotherapy and HDC with ASCT in untreated DLBCL patients with a poor prognosis. Here, we report the results of a prospective phase II trial, and compare the results with a historical cohort of patients that were treated in the pre-Rituximab era in a previously published phase II study.18

Design and Methods

Rituximab-HDC phase II study

The Rituximab-HDC study was a phase II multicenter trial for the treatment of young patients with DLBCL with a poor prognosis conducted by the GIMURELL. From June 2002 to December 2005, 97 consecutive patients were enrolled (R-HDC study group). The study, registered at http://www.clinicaltrials.gov, under study NCT00556127, was performed in accordance with the Helsinki declaration and approved by the ethics review committee of all participating centers. All patients gave written informed consent.

Patients

The inclusion criteria were: previously untreated aggressive B-cell lymphoma (DLBCL), primary mediastinal lymphoma, follicular grade II/III lymphoma; 19 age 18–60; III–IV Ann Arbor stage; 0-2 Eastern Cooperative Oncology Group performance status (PS); intermediate/high (IH) and high (H) risk score according to aa-IPI. The exclusion criteria were: major organ dysfunction; HIV, hepatitis B or C virus seropositivity; central nervous system (CNS) involvement at diagnosis. Histological diagnoses of all patients were reviewed at Pathology Department of the University of Torino by DN. The mandatory baseline assessment included: physical examination; chest and abdomen CT scans; bone marrow (BM) biopsy; full laboratory workup and MUGA scan 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. Patients were retrospectively classified according to the Revised International Prognostic Index (R-IPI).19

Treatment plan

The trial design is shown in Table 1. The treatment consisted of three phases: (i) an induction phase lasting two months with four courses of a dose-dense chemotherapy Rituximab-MegaCEOP14 at two-week intervals with granulocyte-colony-stimulating factor (G-CSF) support; (ii) an intensification phase with two cycles of high-dose chemoimmunotherapy Rituximab-MAD every 28 days with G-CSF;18 two doses of Rituximab 375 mg/m2 were administered on day 4, as well as prior to peripheral blood stem cell (PBSC) harvest during the first MAD course as an in vivo purging; (iii) a consolidation phase consisting of myeloablative chemotherapy according to the BEAM regimen, 21 followed by ASCT with at least 5×10^6 peripheral blood CD34+ cells/kg body weight.

At the end of the treatment, Involved Field Radiotherapy (IF-RT) 25-30 Gy was planned to be administered to areas of previous bulky disease. Patients with BM, hard palate, orbit or paranasal sinus involvement received CNS prophylaxis with four doses

<table>
<thead>
<tr>
<th>Phase</th>
<th>R-HDC</th>
<th>MACOP-B</th>
<th>HDC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Induction (months 1 and 2)</td>
<td>R-MegaCEOP14</td>
<td>4 cycles every 14 days</td>
<td>8 weekly infusions</td>
</tr>
<tr>
<td>Intensification (months 3 and 4)</td>
<td>R-MAD</td>
<td>2 cycles every 28 days</td>
<td>MAD</td>
</tr>
<tr>
<td>High dose chemotherapy (month 5)</td>
<td>Rituximab: day 4 after 1 MAD and prior to PBSC harvest</td>
<td>1 cycle</td>
<td>BEAM + ASCT</td>
</tr>
</tbody>
</table>

R: Rituximab (375 mg/m2); MC: MegaCEOP etoposine (110 mg/m2), cyclophosphamide (1200 mg/m2), vincristine (1 4 mg/m2, maximum 2 mg) day 1, prednisone (40 mg/m2) orally days 1 to 5. Granulocyte-colony-stimulating factor (G-CSF 5 µg/Kg/day) subcutaneously days 3 to 9; MAD mitoxantrone (8 mg/m2/day), high-dose cytarabine (2 g/m2/12 hours for six doses in three hour infusions), dexamethasone (4 mg/m2) 24 hours after the last dose of high-dose cytarabine until Peripheral Blood Stem Cell (PBSC) harvest or recovery from neutropenia; BEAM: cytarabine (300 mg/m2) day -7, etoposide (100 mg/m2/12 hours), cytarabine (200 mg/m2/12 hours) days -6 to -3, melphalan (140 mg/m2) day -2. Autologous Stem Cell Transplantation (ASCT) day 0, MACOP-B21.
of 12 mg intrathecal methotrexate during the induction phase. Supportive care during the intensification and the consolidation phase was given according to local guidelines.

**Historical comparison: HDC group**

The R-HDC study group was retrospectively compared to 41 consecutive DLBCL patients enrolled by the same cooperative Group between August 1991 and August 1995 into a phase II trial of HDC and ASCT without Rituximab, (HDC historic control group). This study was previously reported elsewhere. Patients from the control group with a T-cell phenotype or a histological subtype other than DLBCL or PML were excluded. The inclusion/exclusion criteria and the staging of the HDC historic control group study were the same as those of the R-HDC study group.

Treatment in the HDC historic control group also consisted of three phases (Table 1): (i) an induction phase with eight weeks of MACOP-B chemotherapy; (ii) an intensification phase composed of two courses of the MAD regimen that was identical to the study group, except for the absence of Rituximab; (iii) a consolidation phase with BEAM followed by ASCT with PBSC. The criteria for CNS prophylaxis, radiotherapy and stem cell harvesting were the same as in the R-HDC study group.

**Assessment of response**

In both studies, response assessment was performed one month after the end of the program by the treating physician according to the criteria by Cheson et al. No response was defined as any response less than a Partial Response (PR), stable disease, progressive disease or any death during treatment period.

**Study design and statistical methods**

According to the evidence available when the R-HDC study was planned, the sample size was calculated using a Fleming’s single-stage design using the failure-free survival (FFS) as the principal endpoint. Given the FFS of 50% at three years with the HDC regimen, the sample size was calculated in order to show at least a FFS of 5% with the new R-HDC treatment, with an alpha error of 0.20; the beta error of 0.20; the required sample size was 85 (actually, 97 patients were enrolled to take into account 10% losses to follow-up). All the enrolled patients were considered assessable and results were analyzed on an intention-to-treat basis.

The FFS includes all patients with treatment failure defined as progression at any time during treatment, less than a Complete/Complete undefined Response (CR/CRu) at the end of treatment, relapse or death from any cause. The overall survival (OS) includes all patients, with an event defined as the death of patient due to any cause. The FFS and OS were calculated from the date of diagnosis to the date of failure or death or the last follow-up without any event and reported with 95% confidence intervals (95% CI).

Comparisons between study and historic control group characteristics were performed using the chi-square test or Fisher’s exact test; means of continuous variables were compared by two-sided t-tests. To improve the comparison with the historical control group, all OS and FFS times were censored at the 60th month of follow-up or at the date of last contact. All curves were plotted according to Kaplan and Meier method24, and evaluated by the log-rank test. The Cox proportional hazard model was used to evaluate the effect of R-HDC treatment. The hazard ratios (HR) and corresponding 95% CIs were adjusted for unbalanced or important prognostic factors (age, aa-IPI, BM involvement, bulky disease, number of extranodal sites and B symptoms). A subgroup analysis was performed for FFS and OS using a statistical test for the interaction between the aa-IPI score and treatment. All calculations were performed using the SAS (v. 8.2) package.

**Results**

**Rituximab-HDC phase II study Patient characteristics**

Ninety-seven consecutive patients were enrolled. Three patients were excluded because of change of diagnosis at central pathology review (two follicular grade 3a and one mantle cell blastoid variant). Ninety-four fulfilled the inclusion criteria and were included into the R-HDC study group. The median age was 47 years (range 19-60). The clinical characteristics of the patients are listed in Table 2.

According to aa-IPI, 50 patients (53%) were at intermediate/high and 44 (47%) at high risk score; according to R-IPI, 34 patients (36%) were at score 2 and 60 (64%) at score 3-4.

**Feasibility of the treatment**

Seventy-six (81%) of the 94 patients completed treatment and underwent ASCT. Reasons for not completing the planned treatment in the remaining 18 patients included disease progression in nine patients, toxic death in four, pancreatic hemorrhage in one, and inadequate stem cell collection in four patients (Figure 1). Thirty-two (34%) patients were delivered IF-RT to previous area of bulky disease after completion of the chemotherapy.

**Response to treatment and outcome**

Seventy-seven patients (82%, 95% CI: 73%-88%) achieved a CR/CRu and one experienced PR. Progressive disease was documented in 11 patients (12%) of whom four had CNS progression before ASCT and none of them had received intrathecal prophylaxis; five patients (5%) died of toxicity.

The median follow-up for censored patients was 49 months. Twenty-four R-HDC patients (26%) failed during the first four years and 18 patients (19%) died. The four-year FFS rate was 75% (95% CI: 63.5%-82.5%) and the four-year OS rate was 80% (95% CI: 71.6%-88.4%) (Figure 2). Of the 24 failures, 11 had progressive disease during treatment and died of lymphoma; five patients died of acute toxicity, one patient in PR who progressed early after treatment, is currently alive after...
second line chemotherapy. Seven patients relapsed: five of them are alive after different salvage treatments and two died of lymphoma.

Subgroup analyses according to the aa-IPI and R-IPI for survival were: four-year OS aa-IPI score 2 87%, aa-IPI score 3 73%; four-year OS R-IPI score 2 87%, R-IPI score 3-4 76%, respectively.

Hematological engraftment and safety

All 76 patients who underwent ASCT achieved a complete hematological engraftment. The median times to recovery of an absolute neutrophil count (>0.5×10^9/L) and of a self-sustaining platelet recovery (>50×10^9/L) were 9 days (3-27 days) and 13 days (1-72 days), respectively.

The hematological toxicity was mild during the R-MegaCEOP induction phase: according to the World Health Organisation (WHO) toxicity criteria grading system, a grade 3 or higher hematological toxicity for neutrophils was recorded in 30% of the total number of R-MegaCEOP courses delivered and for platelets and hemoglobin occurred in less than 10% of the courses. During R-MAD and BEAM with ASCT, transfusional support was as follows: median 2.2 and 5 platelet concentrates, and median 1.4 and 3 packed red cell transfusions for the R-MAD and BEAM, respectively.

Severe non-hematological toxicities with a WHO grade >3 were reported in 50 patients (53.2%; 95%CI: 43.2–63.0) (Table 3). As expected, mucositis and gastrointestinal toxicity were frequently observed during the myeloablative phase. No cardiac, renal and hepatic events were recorded. Overall, 18 (19%) episodes of acute severe infections were reported in the 94 R-HDC patients. Five patients died of toxicity during treatment: three patients died of Escherichia coli sepsis; one of Staphilococcus pneumonia and one died of Pseudomonas aeruginosa pneumonia (Figure 1).

Two late infections occurred, at 11 and 13 months after treatment: one severe disseminated herpes zoster virus infection and one bacterial meningitis infection. Both patients recovered completely. So far, no cases of secondary acute myelogenous leukemia (AML), myelodisplastic syndrome (MDS) or solid tumor have occurred.

Historical comparison

The R-HDC study group was compared to the HDC historical group. As listed in Table 2, patients treated

| Table 2. Clinical characteristics of the patients, by study group. |
|------------------------|------------------------|------------------------|------------------------|
| Characteristic         | R-HDC: n=94 (No. of patients (%)) | HDC: n=41 (No. of patients (%)) | Fisher’s Test |
| Age, years             | Median (range) 47 (19-60) | 46 (19-59) | n.a. |
| Gender                 | Male 53 (56) | 26 (63) | 0.569 |
|                       | Female 41 (44) | 15 (37) | |
| Histological sub-types | Diffuse large B-cell 81 (86) | 35 (85) | 0.428 |
|                       | Primary mediastinal B-cell 10 (11) | 6 (15) | |
|                       | Follicular B cell grade 3b 3 (3) | 0 (0) | |
| B symptoms             | Absent 49 (52) | 17 (41) | 0.492 |
|                       | Present 45 (48) | 24 (59) | |
| Performance status grade | 0-1 34 (36) | 15 (37) | 1.00 |
|                       | >1 60 (64) | 26 (63) | |
| Ann Arbor stage        | III 21 (22) | 9 (22) | 1.00 |
|                       | IV 73 (78) | 32 (78) | |
| No. extranodal sites   | 0-1 61 (65) | 19 (46) | 0.056 |
|                       | >1 33 (35) | 22 (54) | |
| Bone marrow involvement | Absent 68 (72) | 23 (56) | 0.074 |
|                       | Present 26 (28) | 18 (44) | |
| Tumor bulk             | Absent 52 (55) | 16 (39) | 0.094 |
|                       | Present (> 10 cm) 42 (45) | 25 (61) | |
| Age-adjusted IPI risk  | Intermediate-high 50 (53) | 17 (41) | 0.262 |
|                       | High 44 (47) | 24 (59) | |

n.a. not applicable
with R-HDC were comparable to those treated with HDC, except that a lower percentage of R-HDC patients exhibited involvement of more than one extranodal site (35% vs. 54%; \( p = 0.056 \)). However, the distribution of patients into aa-IPI subgroups (IH and H risk) did not differ between the R-HDC and HDC groups.

In the HDC historic control group, 31 (76%) of the 41 patients completed treatment and underwent ASCT. The reasons for not completing the planned treatment in the remaining 10 patients were: disease progression in seven patients, toxic death in two patients, and inadequate stem cell harvest in one. Ten (24%) patients were given IF-RT after completion of the chemotherapy with no difference with the R-HDC group (\( \chi^2 = 0.265 \)).

The median follow-up for censored patients was 72 months for the HDC group and 49 months for the R-HDC group. Due to differences in the length of follow-up between the two studies, the outcome comparisons were made at four years to ensure comparable follow-up times.

The four-year FFS rate was: 73% for the R-HDC group and 44% for the HDC control group, with a crude HR of 0.39 (95% CI= 0.22-0.69, \( p = 0.001 \)) (Figure 3a). The actuarial OS rate at four years was 80% for the R-HDC group and 54% for the HDC group, with a crude HR of 0.37 (95% CI= 0.19-0.71, \( p = 0.002 \)) (Figure 3b).

A Cox model was performed to adjust the comparison of treatments for potential confounders such as age, aa-IPI, BM involvement, bulky disease, number of extranodal sites and B symptoms. This analysis confirmed that the risk of failure or death was significantly reduced in the R-HDC group; the adjusted HR for FFS (R-HDC vs. HDC) was 0.44 (95% CI=0.24–0.81, \( p=0.009 \)), and the adjusted HR for OS (R-HDC vs. HDC) was 0.46 (95% CI=0.22–0.90, \( p=0.023 \)).

Subgroup analyses according to the aa-IPI confirmed a better outcome for both IH and H patients treated with R-HDC (four-year FFS aa-IPI 2: R-HDC 80%, HDC 53%; four-year FFS aa-IPI 3: R-HDC 64%, HDC 37%; four-year OS aa-IPI 2: R-HDC 87%, HDC 59%; four-year OS aa-IPI 3: R-HDC 73%, HDC 50%). The statistical tests for interaction did not indicate any meaningful effects upon the advantage of R-HDC versus HDC treatment by aa-IPI subgroups neither for the FFS (\( p \) for interaction term=0.565) nor on the OS advantage (\( p \) for interaction term=0.402).

**Discussion**

The aim of this multi-center, prospective phase II trial was to assess the potential benefit of adding Rituximab to a dose-dense chemotherapy regimen followed by HDC and ASCT in untreated DLBCL patients with a poor prognosis (i.e. aa-IPI IH and H). The results demonstrate that Rituximab-HDC is effective as a first line treatment in a large cohort of patients with a poor prognosis with a prolonged and adequate follow-up. The CR rate was high (82%) and the long-term outcome was also highly favourable with four-year FFS and OS rates of 73% and 80%, respectively.

Some trials have been reported where Rituximab was administered to relapsed patients before and after ASCT in aggressive and follicular lymphoma. The results indicate that this approach is safe and possibly effective.\(^{26-29}\) However, so far, few data have been reported that describe the results of HDC and ASCT supplemented with Rituximab as first line treatment in...
high-risk DLBCL and mainly as abstract forms.\textsuperscript{28,30,31,32} The feasibility of this approach is a major issue when setting up intensified regimens with autografting, and HDC with ASCT programs yielded better results in studies where the drop-out rate of patients was less than 25%.\textsuperscript{10} Our R-HDC program was feasible in a multi-center setting with a drop-out rate limited to 19%. This low rate may have further contributed to the positive outcome of this study.

The impact of Rituximab on hematological and non-hematological toxicities in lymphoma patients undergoing ASCT has been controversial. Rituximab was reported to affect hematological engraftment after ASCT in some studies, but not in more recent ones.\textsuperscript{26,27,33,34} Indeed, we did not observe a delay in platelet or neutrophil engraftment in our R-HDC study.

With respect to non-hematological toxicities, concerns have been raised regarding increased infection rates in Rituximab-treated patients, while other researchers did not confirm these data.\textsuperscript{28,29,35} In our study, the incidence of acute toxicities was as expected. The rate of fatal infections was not negligible, and five patients died of bacterial infections. This figure is similar to the one observed in a recent meta-analysis including 15 randomized trials in 2728 patients with aggressive non-Hodgkin’s lymphoma treated with HDC and ASCT or conventional chemotherapy in the pre-Rituximab era, in which the treatment mortality rate was 5.7% in patients receiving HDC.\textsuperscript{11} In our Rituximab-HDC study, two patients developed late infections one year after ASCT. Overall, these data indicate that patients treated with dose-dense chemotherapy and/or HDC supplemented with Rituximab require adequate anti-microbial prophylaxis and prolonged close clinical surveillance to avoid infections. Notably, no incidences of secondary malignancies have been recorded thus far in our R-HDC group.

The results of our study compare favorably with two recent trials performed in aggressive lymphomas with a poor prognosis treated with chemotherapy schedules characterized by early dose intensification and autografting, but without Rituximab. Patients in these studies experienced a five-year FFS that ranged from 56% to 62%.\textsuperscript{7,36}

To further validate our observations, in addition we compared the results of our R-HDC study with those achieved in a historic group of patients treated with HDC and ASCT without Rituximab. With the limits of a retrospective, non-randomized comparison, our results suggest that Rituximab-HDC scheme may improve the outcome of DLBCL patients with a poor prognosis compared to traditional HDC without Rituximab. Some limits of this historical comparison should be highlighted, which include minor differences in patients populations and in the first part of chemotherapy as well as different follow-up times between the two studies. In order to minimize these differences, the comparison was limited to 4-year follow-up time and adjusted for several potential confounders. The benefit of the R-HDC regimen was...
shown in a multivariate analysis after adjustment for age, aa-IPI, BM involvement, bulky disease, number of extranodal sites and B symptoms. The risk of failure or death was confirmed to be significantly reduced in the R-HDC group by more than 50% for both FFS and OS. Moreover, the improvement for patients treated with R-HDC occurred in both the IH and H aa-IPI groups. Nevertheless, the benefit observed with the new scheme may not be only attributed to the addition of Rituximab but to the whole new scheme.

The efficacy of R-MegaCHOP + R-MAD + BEAM and ASCT may be explained by the rapid tumor reduction during the first part of dose-dense chemoimmunotherapy, and by the addition of a non-cross resistance high-dose cytarabine chemotherapy supplemented with Rituximab (R-MAD) that further increases the response rate and avoids the onset of resistant clones. Indeed, in the R-HDC group, only 12% of the patients were refractory to treatment and progressed during therapy. It is noteworthy that this improvement occurred in patients with an aa-IPI score of 2 and 3. These patients usually show a relevant proportion of refractoriness when treated either with conventional treatment or HDC and ASCT without Rituximab. A more intensive induction therapy before ACST, as applied into our study, may play a favorable role to improve the outcome of poor-prognosis aggressive lymphomas, even without Rituximab, as suggested by the encouraging results reported in a prospective trial with CHOP followed by a dose-intensive cyclophosphamide, etoposide, cisplatin cycle and high dose chemotherapy BEAM with ASCT.

Nowadays the current standard therapy for advanced stage DLBCL is Rituximab-CHOP chemotherapy as shown by randomized trials conducted either in elderly or in young low-risk patients and by a historical comparison of population-based study. The appropriate therapy for young patients with IH and H risk DLBCL is still a subject of debate. Several phase II non-randomized studies incorporating Rituximab into dose-dense or dose-intense schemes, namely R-CHOP14, but without ASCT showed that such approaches are feasible and likely effective in high-risk young DLBCL patients. However from these studies it is difficult to have an estimate of the outcome of young patients with poor prognosis that were analyzed as a subgroup. Overall, the reported two or five-year PFS for patients with aaIPI intermediate-high or high risk score ranged from 45% to 61% suggesting that 40-50% of these patients are unlikely to be cured by standard R-CHOP. Recently, a Revised International Prognostic Index (R-IPI) was retrospectively applied to patients with DLBCL treated with R-CHOP distinguishing three separate prognostic groups with different 4-year OS rates: very good risk 94%, good risk 79% and poor risk 55%. We retrospectively classified our patients according to R-IPI and 36% were at good risk and 64% at poor risk with 4-year OS rates of 87% and 76% respectively. Although our data are not strictly comparable because we included only patients less than 60 years, the results here reported are encouraging and support further studies to evaluate the efficacy of R-HDC with ASCT compared with RCHOP-like regimens into randomized trials, namely in the group with poor prognosis.

Intensified chemoimmunotherapy with HDC and ASCT is one possible strategy to treat DLBCL with poor prognosis. Alternatively, the evaluation at diagnosis of biological markers or models such as gene expression profiling, microvascular density and others may allow to better identify patients that are likely to fail R-CHOP alone. The interim evaluation of response with early positron emission tomography (PET-CT) imaging has shown promise as a prognostic factor in retrospective studies of DLBCL, but requires further investigation because, unlike Hodgkin’s disease, contradictory results have been reported in DLBCL. Both issues are worthwhile areas of future researches that need to be tested into prospective trials.

In conclusion, the encouraging results here reported suggest that R-HDC and ASCT approach may be effective in young DLBCL patients with a poor prognosis. However, the issue if Rituximab-HDC may be more effective compared with Rituximab-dose-dense chemotherapy in these patients will be addressed only by randomized phase III trials that are currently ongoing into the major cooperative groups such as Groupe d’Etude des Lymphomes de l’Adulte and North American Intergroup Study and others. The results of the present study have provided the rationale for the ongoing, prospective, phase III randomized trial, conducted by the Italian Lymphoma Intergroup (registered at http://www.clinicaltrials.gov: NCT00499018), that tests the potential benefit of adding Rituximab to HDC compared with dose-dense chemoimmunotherapy without ASCT to better define the proper therapy for young DLBCL patients with a poor prognosis.

**Authorship and Disclosures**

UV conceived and designed the trial, performed research as the principal investigator and wrote the paper. All authors contributed to writing the paper and checked the final version. UV, EA, GR, AML, MGC, VP and AL planned the study and wrote the study protocol; DN performed the pathology review; AC and BB collected and checked the accuracy of the data; GC performed the statistical evaluations; GG, LF, RF, LO, EP, DRS, FS, ATo and ATu were co-investigators in performing the research, treating and documenting patients and editing the manuscript.

The authors reported no potential conflicts of interest.
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