Lenalidomide plus cyclophosphamide, doxorubicin, vincristine, prednisone and rituximab is safe and effective in untreated, elderly patients with diffuse large B-cell lymphoma: a phase I study by the *Fondazione Italiana Linfomi*

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ABSTRACT

Despite improvements in standard therapy with rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone for patients with untreated, diffuse large B-cell lymphoma, up to 40% of these patients relapse. Lenalidomide alone or in combination with rituximab has been shown to be active in relapsed/refractory aggressive lymphomas. In this phase I study we determined the maximum tolerated dose of lenalidomide plus rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone in untreated, elderly (median age 68 years) patients with diffuse large B-cell lymphoma. Four lenalidomide doses (5, 10, 15, and 20 mg/day on days 1-14) allocated using the continual reassessment method were planned to be administered for 14 days in combination with each course of rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone for a total of six courses. Seven cohorts of patients (n=3 in each cohort) were treated (total n=21) at 10, 20, 15, 15, 15, 10, and 10 mg of lenalidomide. Dose-limiting toxicities occurred in seven patients during the first three courses of treatment. The third dose-level of lenalidomide (15 mg/day) was selected as the maximum tolerated dose, with an estimated probability of dose-limiting toxicities of 0.345 (95% credibility interval 0.164-0.553). Grade 3-4 hematologic adverse events were: neutropenia in 28% of the courses, thrombocytopenia in 9%, and anemia in 3%. Non-hematologic toxicities were moderate: grade 4 increase of creatinine phosphokinase (n=1), grade 3 cardiac (n=2), grade 3 neurological (n=3), and grade 3 gastrointestinal (n=1). In this phase I study, the overall response rate was 90%, with 81% achieving complete remission. This combination regimen appears safe in elderly patients with diffuse large B-cell lymphoma and its efficacy will be assessed in the ongoing phase II trial. *This trial was registered at www.clinicaltrials.gov as NCT00907348.*

Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin's lymphoma, and subjects aged over 60 years account for more than half of all patients newly diagnosed with DLBCL.[1] Over the past decade there have been significant improvements in long-term disease control and survival in patients with DLBCL, with over half of patients maintaining remissions beyond 5 years. This is largely due to the routine incorporation of rituximab into the standard anthracycline-based chemotherapy regimen of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP).[2-7] Based on these and other studies, rituximab-CHOP (R-CHOP) has become the standard of care therapy for patients with newly diagnosed DLBCL. The 3-weekly R-CHOP regimen (R-CHOP21) used in the LNH98-5 trial for elderly patients resulted in fewer patients with disease progression and subsequent lower rates of relapse compared with that achieved by CHOP21.[2] In a recently completed phase III study, biweekly R-CHOP did not improve overall survival or progression-free survival compared with standard R-CHOP21 in elderly patients newly diagnosed with DLBCL.[8] R-CHOP21 is, therefore, the best current standard chemotherapy regimen for these patients.

However, up to 40% of all patients do not respond to initial therapy or they relapse.[2,5] An option to improve the prognosis of high-risk patients may be to administer high-dose chemotherapy followed by consolidation with stem cell

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transplantation, but the results to date are contradictory and this option is limited only to young patients.[2,9-11] Indeed, as the median age of patients diagnosed with DLBCL is in the mid-60s,[1,12,13] many older individuals and others with comorbidities are in particular need of better initial therapy because they cannot tolerate intensive salvage chemotherapy with stem cell transplantation.[14] Novel therapeutic approaches beyond rituximab are, therefore, still required.

Lenalidomide has a complex mechanism of action that involves immune modulation,[15] anti-angiogenesis,[16] modulation of the microenvironment, restoration of immune synapses,[17] and direct antitumor effects.[18] Lenalidomide monotherapy exhibits significant activity in patients with relapsed aggressive B-cell lymphomas, including DLBCL[11,19] and mantle cell lymphoma.[20] Moreover, lenalidomide has been investigated in the treatment of patients with relapsed/refractory DLBCL as part of a combination regimen in a case report,[21] and in combination with rituximab in a phase II study.[22] The novel mechanisms of action of lenalidomide, which are distinct from those of both traditional chemotherapy and rituximab, combined with the *in vitro* synergy of lenalidomide with rituximab and cytotoxic therapy,[23,24] provide a rationale for the addition of lenalidomide to initial R-CHOP therapy in aggressive B-cell lymphomas. In two recent phase I studies, lenalidomide was safely combined with R-CHOP21 in patients with untreated non-Hodgkin's lymphomas.[25,26]

On this basis, the *Fondazione Italiana Linfomi* designed the current phase I/II study to evaluate the safety, tolerability, and efficacy of lenalidomide when administered with R-CHOP21 (LR-CHOP21) in elderly patients with untreated DLBCL or follicular lymphoma (FL) grade IIIb. Here, we report the results from the phase I part of the study.

Methods

This open-label, multicenter phase I/II study included patients aged 60 to 80 years with newly-diagnosed CD20-positive DLBCL or FL grade IIIb who gave written informed consent. The trial was performed in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines; ethical approval was obtained from the independent Ethics Committees and Institutional Review Boards of each site.

Phase I was designed as an open-label, dose-escalation study to determine the maximum tolerated dose (MTD) of lenalidomide administered on days 1 to 14 in combination with standard dose R-CHOP21 chemotherapy [375 mg/m^2 rituximab, 750 mg/m^2 cyclophosphamide, 50 mg/m^2 doxorubicin, 1.4 mg/m^2 (capped at 2.0 mg) vincristine, all on day 1; 40 mg/m^2 prednisone on days 1-5] as 21-day cycles for six courses (Figure 1). Lenalidomide dose-escalation levels were 5, 10, 15, and 20 mg/day.

Mandated prophylaxis comprised granulocyte-colony stimulating factors as primary prophylaxis for neutropenia,[27] a low-molecular-weight heparin for deep-vein thrombosis, cotrimoxazole or pentamidine aerosol for *Pneumocystis jirovecii*, and lamivudine in occult hepatitis B virus carriers. Patients at risk of central nervous system involvement[28] were given four doses of methotrexate 12 mg intrathecally. Patients with a bulky tumor mass, systemic symptoms, compressive disease, or rapidly progressive adenopathies were allowed pre-study treatment with steroids and/or a single dose of vincristine 1.4 mg/m^2 (capped at 2 mg) in the 7 days prior to the start of study treatment; in that case vincristine was omitted during the first course of LR-CHOP21.

The primary end-point of phase I was to determine the MTD and dose-limiting toxicities (DLT). Secondary end-points included complete response and overall response rates. Response was assessed after the first three courses of therapy, and patients with complete response or partial response received a further three courses of therapy at the same schedule and doses. Follow-up continued until disease progression, withdrawal at the patient's request, death, or study completion up to 24 months after the end of treatment. Patients with progressive disease or severe toxicity at any time were withdrawn.

The MTD was defined as the dose at which a DLT occurred in 33% of patients and DLT was defined as any grade 3 or higher non-hematologic toxicity, or toxicity resulting in a delay of over 15 days of a planned cycle date observed during the first two courses. After a case of grade 3 motor neurotoxicity in the third course of LR-CHOP21, a protocol amendment in March 2008 called for assessment of DLT during the first three courses.

The continual reassessment method was used to allocate doses.[29] A design with grouped inclusions of three patients per dose level was chosen, with lenalidomide 10 mg/day as a starting dose to be administered to the first cohort. DLT probabilities at all dose levels were updated using Bayes' theorem. The process was repeated until a fixed sample size (n=24) was reached or a stopping criterion was fulfilled[30] using Bayesian Phase I Dose-Ranging software.[31] More details are reported in the *Online Supplementary Material*.

At completion of phase I, a phase II study with Simon's two-stage design was conducted.[32] The results of this study will be reported separately.

Results

Patients' characteristics and disposition

From May 2008 to February 2010, a total of 21 patients (median age 68 years) from eight centers of the *Fondazione Italiana Linfomi* were enrolled into the phase I part of this study. The patients' clinical characteristics are presented in Table 1. Almost all patients (95%) had DLBCL, 81% of patients had an ECOG performance status score of at least 2, and 76% were at intermediate-high or high risk according to the International Prognostic Index.

The first cohort of three patients was allocated and treated with lenalidomide 10 mg/day and they had no DLT. Updated DLT probabilities for the four lenalidomide dose levels associated the highest dose-level with a DLT probability closest to 0.33; therefore, the next three-patient cohort was administered lenalidomide 20 mg/day. Two DLT were observed and on further updating the analysis, dose de-escalation to 15 mg/day was investigated. This dose level was also recommended for the three subsequent cohorts. The four observed DLT up to the fifth cohort of patients suggested lenalidomide 10 mg/day as the dose with the DLT probability closest to 0.33. The two subsequent three-patient cohorts were, therefore, given lenalidomide 10 mg/day and only one DLT was observed. Table 2 lists the assigned lenalidomide dose levels and observed DLT for the 21 enrolled patients.

On final analysis of the entire seven cohorts of patients, the expert committee decided to stop the trial, since the committee considered that three out of the four stopping criteria had been met (see *Online Supplementary Material*). The third dose level of lenalidomide (15 mg/day) was selected as the MTD, with an estimated DLT probability

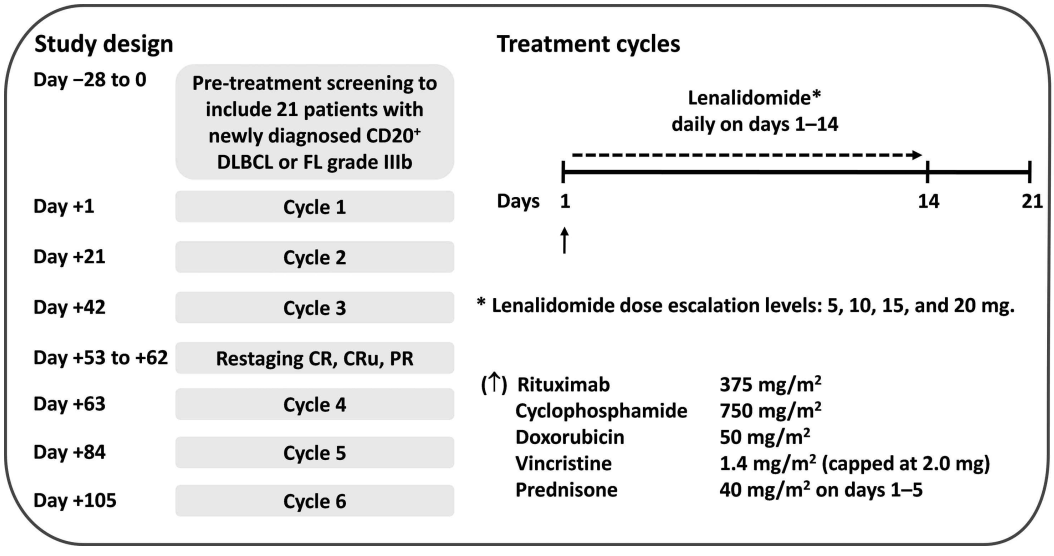

Figure 1. Study design. DLBCL: diffuse large-B-cell lymphoma; CR: complete response; CRu: unconfirmed CR; FL: follicular lymphoma; PR: partial response.

of 0.345 (95% credibility interval: 0.164-0.553). The lenalidomide 15 mg/day dose was in fact the dose with a DLT probability closest to the target fixed rate of 33%; for 15 mg/day the DLT probability was 34% and for 10 mg it was 29%. DLT were recorded in seven patients: two patients at the lenalidomide dose of 20 mg/day; four at the 15 mg/day dose; and one at the 10 mg/day dose. Recorded DLT were: a grade 3 motor neuropathy adverse event (lenalidomide 20 mg/day; cohort 2); grade 3 hypotension during the infusion of rituximab (20 mg/day; cohort 2); a grade 4 increase in creatinine phosphokinase without clinical sequelae (15 mg/day; cohort 3); grade 4 neutropenia that resulted in a delay of the subsequent LR-CHOP21 (15 mg/day; cohort 4); grade 3 motor and sensory neuropathy (15 mg/day; cohort 5); grade 3 neutropenic fever (15 mg/day; cohort 5); and grade 3 diarrhea (10 mg/day; cohort 6).

Feasibility

Of the 126 planned courses of LR-CHOP21, the patients received a total of 117 (93%) courses of R-CHOP21 chemotherapy. Of the 117 courses, 98 (84%) were given with lenalidomide as planned (LR-CHOP21), 12 (10%) with dose and/or day reduction, and seven (6%) without lenalidomide. The median cumulative dose of lenalidomide delivered in nine patients assigned to the 10 mg/day dose was 840 mg [interquartile range (IQR) 460-840 mg], i.e. 100% of the planned dose (840 mg). In the nine patients assigned to the dose of 15 mg/day, the median cumulative dose of lenalidomide was 1185 mg (IQR 1155-1260 mg) i.e. 94% of the planned dose (1260 mg), while in the three patients assigned to the dose of 20 mg/day, it was 1000 mg (IQR 880-1680 mg) i.e. 60% of the planned dose (1680 mg). Lenalidomide therapy was interrupted, reduced, or discontinued during 19 of 117 cycles (16%) in 11 patients. The most frequent cause of lenalidomide reduction or withdrawal was grade 3-4 hematologic toxicity (n=8); other reasons were grade 3 neurological toxicity (n=2) and consent withdrawal (n=1). At least 90% of the planned doses of rituximab, doxorubicin, cyclophosphamide, and vincristine were administered in 93%, 90%, 85%, and 71% of the R-CHOP21 courses, respectively. The median time interval between R-CHOP21 courses was 21 days (range, 19-47).

Table 1. Baseline clinical characteristics of the patients.

Characteristic	N=21
Median age, year (range)	68 (61-77)
Sex, n. (%)	
Male	12 (57)
Female	9 (43)
ECOG performance status score, n. (%)	
0-1	4 (19)
≥2	17 (81)
Ann Arbor stage, n. (%)	
2	4 (19)
3	4 (19)
4	13 (62)
IPI risk score, n. (%)	
2	5 (24)
3	11 (52)
4-5	5 (24)
Lymphoma type, n. (%)	
DLBCL	20 (95)
FL grade IIIb	1 (5)
Bone marrow involvement, n. (%)	6 (29)
Extranodal site involvement, n. (%)	9 (43)
B symptoms, n. (%)	11 (52)
Lactate dehydrogenase level higher than normal, n. (%)	9 (43)
β_2-microglobulin level higher than normal, n. (%)	12 (57)

DLBCL: diffuse large-B-cell lymphoma; ECOG: Eastern Cooperative Oncology Group; FL: follicular lymphoma; IPI: International Prognostic Index.

Three patients received pre-phase treatment with steroids and one dose of vincristine 1.4 mg/m^2 (capped at 2 mg) due to bulky mass in two and rapidly progressive adenopathies in one; in all the three cases vincristine was omitted during the first LR-CHOP21 cycle.

Safety

Granulocyte-colony stimulating factors were administered in 104 (89%) of the 117 cycles: pegfilgrastim in 91 (78%) and lenograstim or filgrastim in 13 (11%).

The most frequently occurring grade 3-4 hematologic toxicity during the 117 treatment cycles was neutropenia

Table 2. Administered lenalidomide doses and estimated probabilities of dose-limiting toxicity (DLT) per dose level.

Cohort	Administered lenalidomide dose (mg/day)	DLT	Updated estimated probability of DLT per lenalidomide dose level (toxicity target 0.33)			
			5 mg/day	10 mg/day	15 mg/day	20 mg/day
1	10	(0, 0, 0)	0.002	0.004	0.007	0.012
2	20	(1, 1, 0)	0.216	0.275	0.329	0.381
3	15	(0, 0, 1)	0.217	0.276	0.331	0.383
4	15	(1, 0, 0)	0.218	0.276	0.331	0.383
5	15	(1, 1, 0)	0.285	0.347	0.403	0.454
6	10	(0, 0, 1)	0.282	0.344	0.400	0.451
7	10	(0, 0, 0)	0.231	0.290	0.345	0.397

(in 28% of the courses), with febrile neutropenia in 5%. Grade 3-4 thrombocytopenia was recorded in 9% of the courses. Except for the grade 4 increase of creatinine phosphokinase, no other grade 4 non-hematologic toxicities were observed. The most frequently occurring grade 3 non-hematologic toxicities were cardiac adverse events in two patients and neurological ones in three patients; no patients died during treatment. Grade 3-4 hematologic adverse events during the 117 treatment cycles and grade 3-4 non-hematologic toxicities in the 21-patient population are summarized in Table 3. These adverse events were transient in nature and manageable. Neurological toxicity of all grades occurred in 13 patients; of these, seven patients experienced grade 1 sensory neurotoxicity, two patients experienced grade 2 sensory neurotoxicity, one grade 2 motor and sensory neurotoxicity, one grade 3 motor neurotoxicity, and two patients experienced grade 3 motor and sensory neurotoxicity. All 13 patients with motor and/or sensory neurological toxicities recovered except one whose toxicity did not disappear but improved to grade 1.

A correlation between neurotoxicity and vincristine administration was analyzed: vincristine was administered at the planned dose in three patients with grade 1 sensory neurotoxicity; the dose was reduced (range 50-75%) in five cases (three grade 1 sensory, one grade 2 sensory, one grade 2 motor and sensory); and was not administered in the other five cases (one grade 1 sensory, one grade 2 sensory, one grade 3 motor, two grade 3 motor and sensory) since the appearance of neurotoxicity. In two cases of grade 3 neurotoxicity (one motor, and one motor and sensory) lenalidomide was reduced or withdrawn.

One patient died 6 months off-therapy due to *Aeromonas hydrophila* sepsis and multi-organ failure while in complete remission.

Clinical response

After six courses of LR-CHOP21 the overall response rate was 90%; 17 of 21 patients (81%) enrolled in this phase I study were in complete remission at the final evaluation, two (9%) were in partial remission, one (5%) had disease progression, and one (5%) was not evaluable for response due to withdrawal of consent after two courses of therapy.

Table 3. All grade adverse events (AE).

AE, n. (%)	Grade 1	Grade 2	Grade 3	Grade 4
Hematologic AE during the 117 cycles of treatment				
Leukocytopenia	9 (8)	17 (15)	21 (18)	3 (3)
Neutropenia	4 (3)	12 (10)	15 (13)	17 (15)
Febrile neutropenia	0	0	5 (4)	1 (1)
Thrombocytopenia	13 (11)	8 (7)	4 (3)	7 (6)
Anemia	32 (27)	7 (6)	4 (3)	0
Non-hematologic AE in the 21-patient population				
Cardiac	0	1 (5)	2 (10)	0
Gastrointestinal	3 (14)	6 (29)	1 (5)	0
Neurological	7 (33)	3 (14)	3 (14)	0
Infective	0	3 (14)	0	0
Other	5 (24)	3 (14)	1 (5)	1 (5)

Discussion

The results from this phase I study show that lenalidomide can be safely administered in combination with the standard R-CHOP21 chemotherapy in patients aged 60 to 80 years with previously untreated DLBCL. Lenalidomide 15 mg/day was identified as the MTD when administered in association with standard R-CHOP21 therapy.

The study's strengths include a rigorous statistical design and the inclusion of only elderly patients with advanced DLBCL. By using a continual reassessment method to allocate doses, fewer patients were required overall, and fewer patients were required to attain the MTD.[29] In this trial no patients were included at the 5 mg/day dose, a few patients were treated at the 10 mg/day dose, and only three patients at the 20 mg/day dose. However, nine patients were treated at the MTD and were, therefore, suitable for analysis in the phase II part of the study.

The 90% overall response rate, with a high percentage of complete responses (81%), suggests that the phase I part of the study is promising. The toxicity profile of this regimen was mild, with no treatment-related deaths and moderate non-hematologic toxicity; only one grade 4 event, an increase in creatinine phosphokinase, was observed. Hematologic toxicity was mild; neutropenia occurred in 28% of the 117 delivered courses (with febrile neutropenia in 5%) and thrombocytopenia in 9% of courses. These figures are close to those reported for prior studies in which similar patients were treated with standard R-CHOP21 with either primary neutropenia prophy-

laxis recommended[5] or not routinely performed, but only if grade 3-4 neutropenia occurred.[33] It is noteworthy that in our trial pegfilgrastim support was recommended because we dealt with elderly patients and there was concern about possible increased myelotoxicity with the addition of lenalidomide to R-CHOP21, as in the studies by Nowakowski *et al.*[25] and Tilly *et al.*[26]

The addition of lenalidomide to R-CHOP is potentially challenging because of the possibility of overlapping toxicities, particularly in an elderly population. The dosing regimen and supporting co-medication program used in the present study were devised to limit these potential negative effects. In this study, the occurrence of at least grade 3 neurological toxicity was possibly more frequent than in patients with DLBCL receiving primary treatment with either CHOP or R-CHOP21.[34] Here, grade 3 or higher neurological toxicity occurred in 14% of patients compared with 5% and 9% of patients treated with R-CHOP or CHOP, respectively.[34] Neurological toxicity may be a matter of concern. A total of 13 patients suffered from neurological toxicity of any grade, but only three patients had grade 3 toxicity and none had grade 4; the toxicity was fully reversible in all except one patient. It is difficult to ascertain whether the addition of lenalidomide increased the neurological toxicity of R-CHOP because this form of toxicity is a fairly common adverse event in elderly patients with DLBCL because of the inclusion of vincristine in the R-CHOP regimen. The most frequent neurotoxicity was sensory, which occurred at a rate similar to that of neurological adverse events due to vincristine. The three grade 3 adverse neurological events were all motor (with or without sensory toxicity): this pattern of motor neuropathy is less commonly seen with vincristine and might possibly be related to the combination with lenalidomide.

The main difference between this study and the study by Coiffier *et al.*[34] is that the number of patients is lower in the present study. It will be interesting to see how the safety data develop further in the ongoing phase II part of this trial in which 49 patients are being studied.

With its activity as a single agent or in combination,[11,15,18-20,35-38] lenalidomide appears to be a strong candidate for inclusion in initial therapy of patients with aggressive B-cell non-Hodgkin's lymphoma. Furthermore, a recent study suggests that lenalidomide has improved activity in activated B-cell-like DLBCL compared with germinal center B-cell-like DLBCL.[39] This issue is a point of interest and an analysis of it is planned in the phase II part of this trial. Two prior phase I studies of LR-CHOP provided initial evidence of clinical activity and good tolerability in patients with advanced untreated DLBCL and FL, but this was in a population not restricted to elderly patients.[25,26] Given the prevalence of DLBCL in the elderly population, we decided to study the LR-CHOP21 regimen in elderly patients with previously untreated DLBCL or FL grade IIIb; indeed in the present study only patients aged 60 to 80 years were eligible and the median age of the participants was 68 years.

The first two studies that investigated the addition of lenalidomide to R-CHOP[25,26] concluded that the MTD was lenalidomide 25 mg for 10 days or 14 days in addition to R-CHOP21; in our trial, the MTD was 15 mg for 14 days. These different results may have a variety of explanations. First, each study enrolled relatively few patients although this is standard in phase I studies. Secondly, in each study patients had different clinical and histological characteristics at diagnosis and thus they may have different tolerances to chemotherapy. Apart from the different ages of the participants, in the present study only patients with DLBCL or FL grade IIIb were included, whereas in the study by Nowakowski *et al.*[25] 83% of patients had DLBCL and 17% had FL grade II or IIIa and in the study by Tilly *et al.*[26] patients with a variety of histological subtypes were enrolled (15% DLBCL, 67% FL, 11% mantle cell, and 7% indolent lymphomas).

Another potentially important explanation of the differences in recommended lenalidomide doses across the three studies is the different statistical analyses used. In our study, we applied the continual reassessment method, which seems to be a more rigorous and less flexible method than standard dose-finding designs. Of note in our study the DLT was defined based on the toxicities observed during the first three courses, whereas in the other two trials the DLT was evaluated on toxicities recorded only during the first course of LR-CHOP21. Our decision was made to avoid and evidence possible cumulative toxicities in subsequent courses of LR-CHOP21, and to allow a more realistic and reproducible schedule of treatment. Finally, based on the study design, we considered all grade 3 extra-hematologic toxicities that occurred, including those not directly related to lenalidomide, such as grade 3 hypotension due to rituximab infusion. This may lead to an overestimation of DLT, but it is always difficult to assess the safety of a multidrug combination and to rule out that the observed toxicity is due to the experimental drug or its potential drug interactions.

It is interesting to note that the cumulative dose of lenalidomide in each course of R-CHOP21 was similar in two studies (Nowakowski *et al.*[25] 250 mg; and the present study 210 mg) but different from the 350 mg in the study by Tilly *et al.*[26] Nevertheless, all three studies underline the feasibility and the potential efficacy of the LR-CHOP21 regimen.

Elderly patients with untreated DLBCL currently have an unmet need for better treatment options because up to 40% of them do not respond to initial therapy or relapse after such treatment.[5,34] The addition of novel drugs to the R-CHOP regimen with biological mechanisms different from chemotherapy, such as lenalidomide and bortezomib, may be a promising strategy to reduce the failure rate of treatment of DLBCL.[40]

In conclusion, this study selected lenalidomide 15 mg/day for 14 days as the MTD to be safely administered in association with the R-CHOP21 regimen. The LR-CHOP21 regimen has shown promising efficacy results (90% overall response rate, including 81% complete responses and 9% partial responses) warranting the conduction of the phase II part of the trial and the design of further studies. Planned biological studies on peripheral blood and on tumor blocks will help clarify the role of lenalidomide in histological subgroups of DLBCL. Randomized trials will be required to fully quantify the potential benefit of the LR-CHOP21 regimen.

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Appendix

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Authorship and Disclosures

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

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