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Lenalidomide Combined With R-CHOP Overcomes Negative Prognostic Impact of Non–Germinal Center B-Cell Phenotype in Newly Diagnosed Diffuse Large B-Cell Lymphoma: A Phase II Study

Grzegorz S. Nowakowski, Betsy LaPlant, William R. Macon, Craig B. Reeder, James M. Foran, Garth D. Nelson, Carrie A. Thompson, Candido E. Rivera, David J. Inwards, Ivana N. Micallef, Patrick B. Johnston, Luis F. Porrata, Stephen M. Ansell, Thomas M. Habermann, and Thomas E. Witzig

Grzegorz S. Nowakowski, Betsy LaPlant, William R. Macon, Garth D. Nelson, Carrie A. Thompson, David J. Inwards, Ivana N. Micallef, Patrick B. Johnston, Luis F. Porrata, Stephen M. Ansell, Thomas M. Habermann, and Thomas E. Witzig, Mayo Clinic, Rochester, MN; Craig B. Reeder, Mayo Clinic, Scottsdale, AZ; and James M. Foran and Candido E. Rivera, Mayo Clinic, Jacksonville, FL.

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Corresponding author: Grzegorz S. Nowakowski, MD, Division of Hematology, Mayo Clinic, 200 First St SW, Rochester, MN 55905; e-mail: nowakowski.grzegorz@mayo.edu.

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ABSTRACT

Purpose
Lenalidomide has significant single-agent activity in relapsed diffuse large B-cell lymphoma (DLBCL). We demonstrated that lenalidomide can be safely combined with R-CHOP (rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone); this new combination is known as R2CHOP. The goal of this phase II study was to evaluate the efficacy of this combination in newly diagnosed DLBCL.

Patients and Methods
Eligible patients were adults with newly diagnosed untreated stages II to IV $CD20^+$ DLBCL. Patients received lenalidomide 25 mg orally per day on days 1 through 10 with standard-dose R-CHOP every 21 days for six cycles. All patients received pegfilgrastim on day 2 of each cycle and aspirin prophylaxis throughout. DLBCL molecular subtype was determined by tumor immunohistochemistry and classified as germinal center B-cell (GCB) versus non-GCB in the R2CHOP patients and 87 control patients with DLBCL from the Lymphoma Database who were treated with conventional R-CHOP.

Results
In all, 64 patients with DLBCL were enrolled, and 60 were evaluable for response. The overall response rate was 98% (59 of 60) with 80% (48 of 60) achieving complete response. Event-free survival and overall survival (OS) rates at 24 months were 59% (95% CI, 48% to 74%) and 78% (95% CI, 68% to 90%), respectively. In R-CHOP patients, 24-month progression-free survival (PFS) and OS were 28% versus 64% ($P < .001$) and 46% versus 78% ($P < .001$) in non-GCB DLBCL versus GCB DLBCL, respectively. In contrast, there was no difference in 24-month PFS or OS for R2CHOP patients on the basis of non-GCB and GCB subtype (60% *v* 59% [$P = .83$] and 83% *v* 75% [$P = .61$] at 2 years, respectively).

Conclusion
R2CHOP shows promising efficacy in DLBCL. The addition of lenalidomide appears to mitigate a negative impact of non-GCB phenotype on patient outcome.

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INTRODUCTION

Approximately 40% of the patients with diffuse large B-cell lymphoma (DLBCL) relapse following initial immunochemotherapy.[1-5] Although intensive high-dose chemotherapy can be used as salvage therapy for some patients with relapsed or refractory DLBCL, the majority will succumb to the disease.[6] The development of a more effective initial therapy is essential for improving long-term outcomes of patients with DLBCL. Before the advent of rituximab, several dose-intensified cytotoxic therapies were introduced, but they failed to provide substantial improvement over the standard anthracycline-based combination of cyclophosphamide, vincristine, doxorubicin, and prednisone (CHOP) or CHOP-like chemotherapy.[7-11] In contrast, the addition of rituximab (a monoclonal antibody with a different mechanism of action than traditional cytotoxic chemotherapy) to CHOP (R-CHOP) significantly improved the results of initial therapy.[5]

Advances in molecular profiling by gene expression profiling (GEP) of DLBCL allowed for the identification of two major DLBCL subtypes: germinal center B-cell-like (GCB) subtype and activated

B-cell-like (ABC) subtype.[12] The ABC subtype is also referred to as non–germinal center B-cell (non-GCB) subtype in immunohistochemistry (IHC) -based classifications.[13] Patients with ABC DLBCL have a significantly worse outcome when treated with R-CHOP or R-CHOP-like chemotherapy[14]; however, no therapy to date has been proven to improve the outcome of patients with ABC DLBCL. Consequently, R-CHOP is considered the standard-of-care therapy in 2014 for patients with advanced newly diagnosed DLBCL, regardless of molecular subtype.

Lenalidomide, an immunomodulatory drug, showed significant activity in relapsed DLBCL.[15-17] Recently, synthetic lethality of lenalidomide in ABC DLBCL has been described, providing insight into the mechanism of activity of lenalidomide in DLBCL that indicates preferential activity in the ABC subtype of DLBCL.[18] In concordance with in vitro findings, the clinical activity of lenalidomide in relapsed and refractory DLBCL appears to be significantly higher in non-GCB DLBCL than in GCB DLBCL.[19]

The mechanisms of action of lenalidomide that are novel and distinct from both traditional chemotherapy and rituximab provide a strong rationale for the introduction of lenalidomide to first-line therapy in DLBCL. We previously demonstrated that lenalidomide 25 mg per day on days 1 through 10 of a 21-day cycle could be safely combined with standard-dose R-CHOP21 (R-CHOP administered over a 21-day cycle).[20] Herein, we report the results of a phase II trial of this combination with an emphasis on efficacy analysis by DLBCL phenotypic subtype.

PATIENTS AND METHODS

Study Design and End Points

This was an investigator-initiated, open-label, single-arm phase II study with the primary end point of event-free survival (EFS). EFS was defined as the time from the date of registration to the date of the first disease progression, subsequent antilymphoma treatment, or death as a result of any cause. Secondary end points were progression-free survival (PFS) defined as the time from the date of registration until the date of disease progression or death as a result of any cause, overall survival (OS) defined as the time from the date of registration until the date of death as a result of any cause, and response rate. All patients had positron emission tomography (PET) combined with noncontrast computed tomography (CT) at diagnosis. Responses were evaluated by PET with CT after two and six cycles of treatment using standard response criteria as published by Cheson et al.[21] Adverse events were defined per National Cancer Institute Common Terminology Criteria for Adverse Events v3.0. Toxicity was defined as an adverse event that was classified as being possibly, probably, or definitely related to study treatment.

Key Eligibility Criteria

Eligible patients were age 18 years or older (there was no upper age limit) with newly diagnosed, untreated, $CD20^+$ stages II to IV DLBCL, and measurable disease was defined as at least one lesion $\geq$ 1.5 cm in a single diameter by CT. Patients were required to have Eastern Cooperative Oncology Group performance status 0 to 2; estimated cardiac ejection fraction $\geq$ 45%; absolute neutrophil count $\geq$ 1,500/μL; platelet count of $\geq$ 100,000/μL; total bilirubin $\leq$ 1.5$\times$ upper limit of normal (ULN) or, if total bilirubin was more than 1.5$\times$ ULN, the direct bilirubin must have been normal; alkaline phosphatase and aspartate aminotransferase $\leq$ 3$\times$ ULN unless there was evidence of direct liver involvement by lymphoma, then $\leq$ 5$\times$ ULN; and creatinine $\leq$ 2$\times$ ULN. Exclusion criteria included pregnant or nursing women, HIV infection, presence of CNS involvement, post-transplantation lymphoproliferative disorder, history of myocardial infarction within the past 6 months, or therapy with erythroid-stimulating agents. Patients with a history of life-threatening or recurrent thrombosis and/or embolism were excluded unless they were receiving anticoagulation therapy during the treatment.

Pathology Review and DLBCL Phenotype Assessment

All pathology was confirmed by central pathology review (W.R.M.) using WHO diagnostic criteria. DLBCL phenotype (cell of origin subtype) was determined by using an IHC method developed by Hans et al[12] that was performed at Mayo Clinic on sections of paraffin-embedded tissue by using antibodies directed against CD10, Bcl-6, and IRF-4 (MUM-1). DLBCLs were regarded as positive for a specific antigen if $\geq$ 30% of the tumor cells were stained for that antigen. Patients who were $CD10^+$ or Bcl-6-positive in the absence of CD10 and MUM1 expression were classified as GCB subtype. Patients who lacked CD10 but expressed MUM1 (regardless of the Bcl-6 staining result) or lacked all three antigens were classified as non-GCB subtype.

Treatment

Lenalidomide orally 25 mg per day was administered on days 1 through 10 of each cycle and delivered concomitantly with standard dose R-CHOP-21 chemotherapy (rituximab 375 mg/m^2, cyclophosphamide 750 mg/m^2, doxorubicin 50 mg/m^2, vincristine 1.4 mg/m^2 [capped at 2.0 mg], all on day 1; prednisone 100 mg/m^2 per day on days 1 through 5). All patients received a pegfilgrastim 6-mg subcutaneous injection on day 2 and low-dose aspirin (acetylsalicylic acid) 81 mg per day prophylaxis throughout, unless they were on therapeutic dose warfarin or low molecular weight heparin for intercurrent conditions. The treatment continued for a maximum of six cycles or until disease progression. Tumor lysis prophylaxis, antiemetics, and supportive care were standard of care and at the discretion of the treating physician.

R-CHOP Control Cohort

All consecutive patients with stage II to IV DLBCL treated with conventional R-CHOP enrolled between February 2004 and March 2010 in an actively maintained prospective Mayo Clinic Lymphoma Database, and who met the same inclusion criteria as those treated with R2CHOP were identified and analyzed for outcome on the basis of DLBCL subtype and had tissue available for pathology review and IHC. All R-CHOP patients had their pathology centrally reviewed. The IHC methods and laboratory (Mayo Clinic) were the same as that used for patients treated with R2CHOP and the same as that used in the previous report of differential activity of lenalidomide in non-GCB subtype in relapsed DLBCL.[19]

Statistical Considerations and Study End Points

This phase II study used a one-stage binomial design to assess the efficacy and tolerability of R2CHOP in patients with DLBCL. The study had 93% power, with a 9% type I error rate. Thirty-two evaluable patients were required to test the null hypothesis that the rate of EFS at 12 months (EFS12) for this regimen is at most 80% versus the alternative hypothesis that the true EFS12 rate is 95% or greater. An event was defined as death as a result of any cause, tumor progression and/or relapse, or initiation of subsequent antilymphoma therapy following R2CHOP study therapy. A 95% binomial CI for the true EFS12 rate was calculated. After the initial 32 evaluable patients were enrolled, data analysis indicated that a higher number of patients at risk (high International Prognostic Index score) had been accrued than initially predicted to calculate the statistical goals. Consequently, a comparison of EFS for patients treated on the study and historical control patients treated with R-CHOP was performed, and it showed a favorable outcome for patients treated with R2CHOP. The data regarding lenalidomide that showed a more pronounced impact on non-GCB DLBCL also became available. After a protocol modification was approved by the institutional review board, additional patients were accrued to further allow the exploratory analysis of the efficacy of R2CHOP by DLBCL subtype. A total of 64 evaluable patients with DLBCL were enrolled and included in this analysis. The distributions of time-to-event end points were estimated by using Kaplan-Meier methods in which differences between groups were evaluated by using log-rank statistics. EFS patients were censored on the date of their last follow-up. Kruskal-Wallis, χ^2, and Fisher's exact tests were used to evaluate differences between cohorts.

Ethics

The study was approved by the institutional review board, and all patients provided written, informed consent to participate in the study.

RESULTS

Between September 2008 and January 2013, 64 patients with DLBCL enrolled and were evaluable (Table 1). The median age was 65 years; 70% of patients were older than age 60 years and 9% were age ≥ 80 years.

Response Rate

Four patients went off study before being evaluated for response (three refused to return to the treatment center for treatment and assessment and one died). The overall response rate in the 60 remaining evaluable patients was 98% (95% CI, 91% to 100%; 59 of 60) with 80% (95% CI, 68% to 89%; 48 of 60) achieving complete response.

EFS, PFS, and OS

Nineteen patients had disease progression and 14 died. Median follow-up in surviving patients was 23.5 months (range, 3.5 to 49.9 months). Median duration of response has not yet been reached. The EFS rate was 70% (95% CI, 60% to 83%) at 12 months and 59% (95% CI, 48% to 74%) at 24 months (Fig 1A).

Table 1. Patient Characteristics

Characteristic	R2CHOP (n = 64) No.	R2CHOP (n = 64) %	Contemporary Cohort of R-CHOP (n = 87) No.	Contemporary Cohort of R-CHOP (n = 87) %	*P*
Age, years					.0132*
Median	65.0		61.0		
Range	22.0-87.0		41.0-86.0		
Sex					.5337†
Female	24	37.5	37	42.5	
Male	40	62.5	50	57.5	
IPI					.0508†
Low	7	10.9	18	20.7	
Intermediate-low	24	37.5	16	18.4	
Intermediate-high	24	37.5	38	43.7	
High	9	14.1	15	17.2	
Ann Arbor stage					.0467†
2	7	10.9	20	23.0	
3	19	29.7	14	16.1	
4	38	59.4	53	60.9	
ECOG PS					.3650‡
0	30	46.9	32	36.8	
1	28	43.8	41	47.1	
2	6	9.4	11	12.6	
3	0	0.0	3	3.4	

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance score; IPI, International Prognostic Index; R-CHOP, rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone; R2CHOP, lenalidomide added to R-CHOP.
*Kruskal-Wallis test.
†χ^2 test.
‡Fisher's exact test.

Because no patients received subsequent treatment for lymphoma before experiencing disease progression, the results for EFS and PFS were identical. The OS rate was 90% (95% CI, 83% to 98%) at 12 months and 78% (95% CI, 68% to 90%) at 24 months (Fig 1B). Three patients developed a second malignancy: one had acute myelogenous leukemia, one had glioblastoma, and one had metastatic colon adenocarcinoma.

Impact of DLBCL Subtype on Outcome

Characteristics of the control patients with DLBCL treated with R-CHOP alone are provided in Table 1. Baseline characteristics were similar between R-CHOP cohorts and patients enrolled onto the R2CHOP study, apart from a higher proportion of patients who were younger and had lower-stage DLBCL receiving R-CHOP. The 2-year PFS in the R-CHOP controls was 52% (95% CI, 43% to 64%; Fig 1C), and the median follow-up for patients still alive was 41.2 months (range, 11.6 to 78.3 months). When analyzed by DLBCL subtype, the 2-year PFS was 28% (95% CI, 15% to 51%) in the non-GCB patients and 64% (95% CI, 53% to 78%) in GCB patients (log-rank $P < .001$; Fig 2A). There was no difference in 2-year PFS of patients treated with R2CHOP on the basis of non-GCB and GCB subtypes (60% [95% CI, 41% to 87%] *v* 59% [95% CI, 44% to 80%], respectively; $P = .83$; Fig 2B). Consequently, PFS of patients with a non-GCB phenotype treated with R-CHOP appeared inferior to that of patients in the R2CHOP group (28% *v* 60%). The difference in PFS based on DLBCL subtype in patients treated with R-CHOP translated to a significant difference in OS. Patients with non-GCB DLBCL treated with R-CHOP had a significantly inferior 2-year OS when compared with patients with GCB DLBCL (46% *v* 78%; $P < .001$; Fig 2C). For patients with a non-GCB phenotype treated with R2CHOP, 2-year OS was not different from that of patients with a GCB phenotype (83% *v* 75%; $P = .61$; Fig 2D). Consequently, the OS of patients with a non-GCB phenotype treated with R-CHOP appeared inferior to that of patients treated with R2CHOP (46% *v* 83% at 2 years).

Toxicity

Hematologic and nonhematologic toxicities during cycles one to six are summarized in Table 2. Grade ≥ 3 nonhematologic toxicities were seen in 25% of patients, with two patients experiencing a grade 4 toxicity. One patient experienced grade 5 sepsis as a result of gut perforation that occurred in an area of DLBCL involvement after first cycle of therapy. For hematologic toxicities, grade ≥ 3 was seen in 94% of patients, with grade 4 in 77%. Grade 3 and 4 neutropenia was present in 13% and 75%, respectively. CBC was monitored weekly for all treatment cycles. Neutropenia was of short duration, and neutropenic complications were rare with only 9% developing grade 3 febrile neutropenia. Grade 3 and 4 thrombocytopenia occurred in 27% and 17% of patients, respectively. Thrombocytopenia was of short duration with rare bleeding complications (1.6%).

Dose Intensity

Eighty-six percent (55 of 64) of patients received all six cycles of R2CHOP therapy. Early discontinuations occurred as a result of refusal to continue participation after cycle 1 (four patients), adverse event (one patient), disease progression (three patients), and death

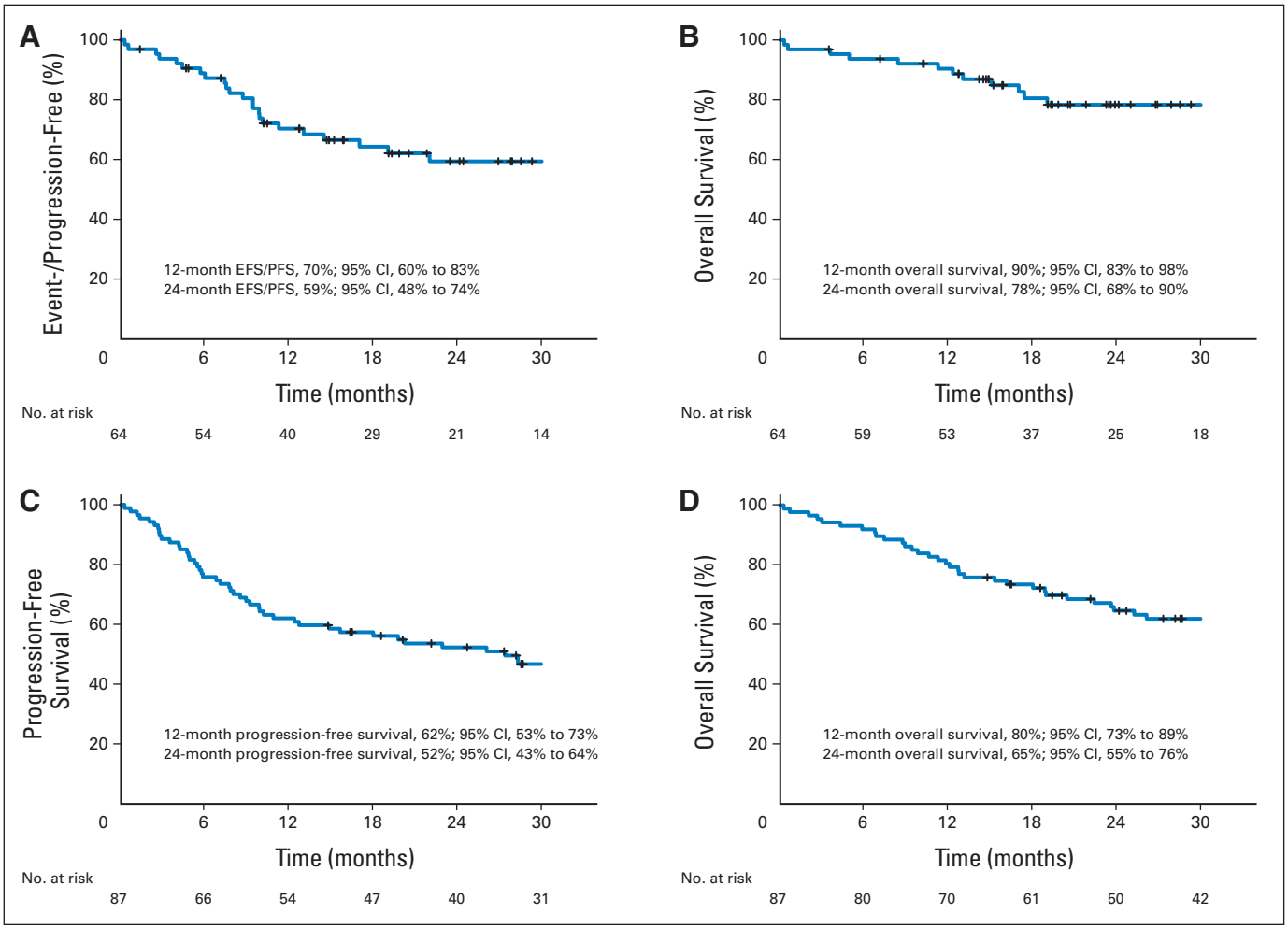

Fig 1. Outcomes of patients treated with R2CHOP (lenalidomide added to R-CHOP [rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone]). (A) Event-free survival and progression-free survival (PFS). Because no patients received subsequent treatment for lymphoma before experiencing disease progression, results for event-free survival and PFS are identical. (B) Overall survival. (C) PFS in R-CHOP historical controls. (D) Overall survival in historical controls treated with R-CHOP.

(one patient). Refusal to participate in all four patients was a result of distance to the treatment center and preference to continue treatment locally with R-CHOP alone. The 64 patients received 356 cycles of treatment. Eighty-seven percent (310 of 356) of cycles contained the full lenalidomide dose. In 6% (20 of 356) of the cycles, lenalidomide was omitted, and 7% (26 of 356) had a lenalidomide dose reduction. In 90% (320 of 356) of the cycles, patients received at least 90% of the intended dose of R-CHOP. Dose reductions of R-CHOP components were prednisone 5% (17 of 356), vincristine 4% (15 of 356), doxorubicin 3% (11 of 356), and cyclophosphamide 3% (eight of 356) of all cycles. When analyzed by number of patients rather than number of cycles, two, four, seven, and nine patients had a reduction of cyclophosphamide, doxorubicin, prednisone, and vincristine, respectively.

DISCUSSION

The clinical benefit of adding rituximab to cytotoxic chemotherapy suggests that improvement in the initial therapy may depend on introducing combinations containing agents with a novel mechanism of action rather than dose intensification of chemotherapy. Lenalidomide has significant single-agent activity and a distinct, pleotropic mechanism of action in DLBCL and therefore is a strong candidate for inclusion in first-line therapy of aggressive B-cell non-Hodgkin lymphoma.[13-15] Lenalidomide demonstrates in vitro synergy with rituximab and cytotoxic therapy[22,23] and may reduce drug resistance.[24,25] We previously conducted a phase I study demonstrating that lenalidomide can be safely combined with R-CHOP21 without an impact on the dose intensity of R-CHOP. Similar phase I studies with slightly different doses and schedules of lenalidomide conducted by Italian (lenalidomide 15 mg per day on days 1 to 14 of R-CHOP21) and French groups (lenalidomide 25 mg per day on days 1 to 14 of R-CHOP21) also demonstrated the feasibility of lenalidomide and R-CHOP combinations.[26,27]

In this phase II study, we evaluated the efficacy of R2CHOP in first-line therapy of DLBCL. The inclusion criteria were designed to enroll patients similar to those seen in clinical practice. Because DLBCL in the elderly is associated with a worse outcome and because

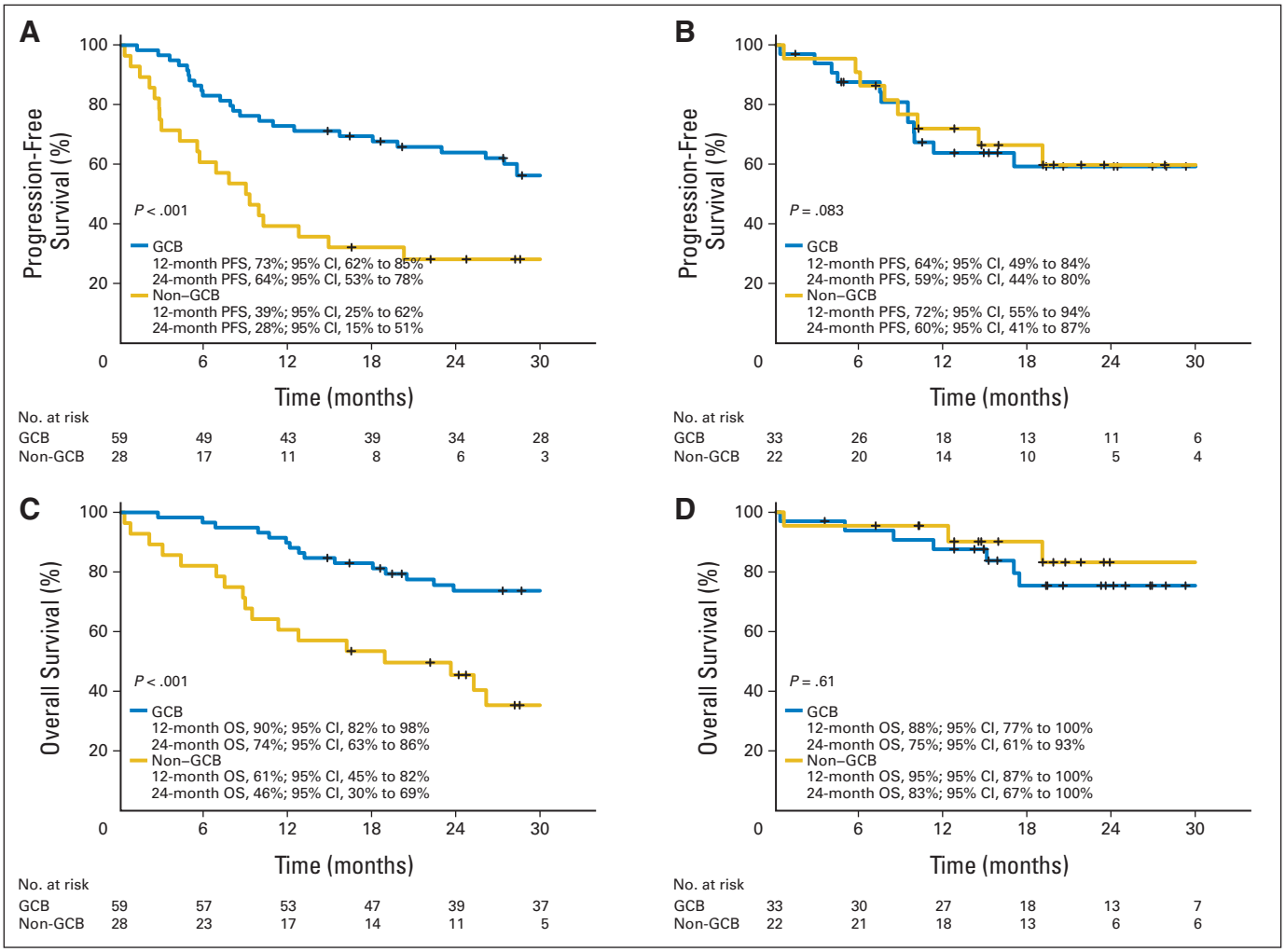

Fig 2. Outcomes of historical control patients treated with R-CHOP (rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone) and study patients treated with R2CHOP (lenalidomide added to R-CHOP) based on germinal center B-cell (GCB) versus non-GCB diffuse large B-cell lymphoma (DLBCL) subtype. (A) Progression-free survival in patients treated with R-CHOP in non-GCB versus GCB DLBCL. (B) Progression-free survival in patients treated with R2CHOP in non-GCB versus GCB DLBCL. (C) Overall survival in patients treated with R-CHOP in GCB versus non-GCB DLBCL. (D) Overall survival of patients treated with R2CHOP in non-GCB versus GCB DLBCL.

elderly patients have limited options for salvage therapy, this study had no upper age limit. Indeed, 70% of patients were age 60 years or older and 9% were age 80 years or older. Response rates evaluated by PET and CT[21] were high, as expected for immunochemotherapy in patients with newly diagnosed DLBCL. The primary end point of the study was EFS. Because no patients received therapy without progression, EFS was the same as PFS in this cohort. EFS and/or PFS in patients treated with R2CHOP is encouraging, considering that 60% of patients had intermediate-high or high International Prognostic Index scores and compared favorably with a historical cohort of patients treated with R-CHOP alone meeting the same inclusion criteria and having similar characteristics.

Toxicity was predominantly hematologic and was similar to expected toxicity from R-CHOP alone. Although neutropenia was common, it was of short duration and was rarely associated with neutropenic complications. Thrombocytopenia was of short duration and was not associated with bleeding complications or the need for platelet transfusion. CBC was monitored once per week for all treatment cycles, likely resulting in reported high incidence of thrombocytopenia and neutropenia in otherwise asymptomatic patients. There was one death related to toxicity for gut perforation following the first cycle of therapy in a patient with known GI involvement by lymphoma. Gut perforation in patients with lymphoma involving GI is a well-recognized complication that is difficult to prevent.[28] Nonhematologic toxicities were infrequent. Grade 2 sensory neuropathy was seen in five (8%) of 64 patients, and one patient experienced grade 3 sensory and motor neuropathy. The incidence of neuropathy appears similar to that reported with CHOP and R-CHOP.[9,29] Lenalidomide use is associated with an increased risk of thrombosis.[30,31] Acetylsalicylic acid has been reported to be an effective prophylaxis and was mandated in this study.[31] With this strategy, only one patient (1.6%) experienced deep vein thrombosis. In this case, lenalidomide was restarted after the patient was given anticoagulation therapy without further problems. A recent meta-analysis estimated the risk of thrombosis in patients with non-Hodgkin lymphoma at 6%[32]; therefore the risk of thrombosis in patients treated with R2CHOP appears not to be

Table 2. Grade 3 and 4 Adverse Events at Least Possibly Related to Treatment (all cycles)

Toxicity	Grade 3		Grade 4		Grade 5	
	No.	%	No.	%	No.	%
Decreased neutrophil count	8	12.5	48	75.0		
Decreased leukocyte count	20	31.3	31	48.4		
Decreased platelet count	17	26.6	11	17.2		
Sepsis (grade 3 to 4 ANC)			1	1.6	1	1.6
Decreased hemoglobin	10	15.6				
Febrile neutropenia	6	9.4				
Dehydration	2	3.1				
Fatigue	2	3.1				
Pneumonia (grade 3 to 4 ANC)	2	3.1				
Decreased serum potassium	2	3.1				
Urinary tract infection (grade 3 to 4 ANC)	2	3.1				
Vascular access complication	2	3.1				
Dyspnea	1	1.6				
Intra-abdominal hemorrhage			1	1.6		
Left ventricular dysfunction	1	1.6				
Decreased lymphocyte count	1	1.6				
Oral mucositis	1	1.6				
Nausea	1	1.6				
Pharyngolaryngeal pain	1	1.6				
Pneumonitis	1	1.6				
Decreased serum albumin	1	1.6				
Decreased serum sodium	1	1.6				
Skin infection (grade 0 to 2 ANC)	1	1.6				
Skin infection (grade 3 to 4 ANC)	1	1.6				
Thrombosis			1	1.6		
Upper respiratory infection (grade 3 to 4 ANC)	1	1.6				
Vomiting	1	1.6				
Weight loss	1	1.6				

Abbreviation: ANC, absolute neutrophil count.

higher than expected. Good tolerability of the R2CHOP regimen was reflected by a large majority of patients who received the intended dose of therapy. Although some patients[4] refused continuation of treatment on the study following cycle 1, all four refusals resulted from patients' preference to receive treatment closer to home and all patients continued R-CHOP therapy alone afterward.

In vitro studies elucidated the mechanism of synthetic lethality of lenalidomide, which occurred preferentially in the ABC subtype of DLBCL.[18] Clinical observations support these laboratory data. In a retrospective analysis of patients with relapsed and refractory DLBCL treated with single-agent lenalidomide, the clinical activity of lenalidomide was significantly higher in patients with non-GCB DLBCL (as defined by the Hans algorithm) than in GCB patients.[19] We therefore performed an exploratory analysis of the outcomes of patients treated with R2CHOP in non-GCB versus GCB subtype. Because the Hans algorithm classification interpretation can be challenging and was not reproduced in all the studies, we applied the Hans classification to the patient cohort treated with R-CHOP as a control to determine whether the Hans algorithm classification predicted outcome in patients treated with R-CHOP. Hans algorithm staining and interpretation was conducted in an experienced pathology laboratory and centrally reviewed for all patients. Indeed, the same laboratory performed Hans algorithm analysis for a significant proportion of patients in a study of relapsed patients with DLBCL, showing preferential clinical activity of lenalidomide in non-GCB DLBCL.[19] As predicted, the outcome of patients with non-GCB DLBCL treated with R-CHOP was significantly inferior to that of GCB patients. R2CHOP was effective in both subtypes; there was no longer any apparent difference in the outcome of patients with non-GCB versus GCB DLBCL. Accordingly, the outcome of patients with non-GCB DLBCL was significantly worse in the R-CHOP cohort than in patients on the R2CHOP study. This suggests that R2CHOP might be particularly active in non-GCB DLBCL, overcoming the negative impact of non-GCB phenotype on outcome.

In conclusion, R2CHOP using a lenalidomide dose of 25 mg per day for 10 days of each cycle is well tolerated and shows promising clinical activity in DLBCL. The addition of lenalidomide appears to be particularly beneficial in non-GCB DLBCL, although the effect in the GCB subtype is less apparent. These findings need to be validated in a randomized study, preferably using GEP in addition to the IHC classification of DLBCL. In this regard, a randomized phase II study of R-CHOP versus R2CHOP using GEP classification led by Eastern Cooperative Oncology Group (E1412; NCT01856192) is currently ongoing.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) and/or an author's immediate family member(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.
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AUTHOR CONTRIBUTIONS

Conception and design: Grzegorz S. Nowakowski, Betsy LaPlant, William R. Macon, James Foran, Stephen M. Ansell, Thomas M. Habermann, Thomas E. Witzig
Provision of study materials or patients: Grzegorz S. Nowakowski, Craig B. Reeder, James Foran, Carrie Thompson, Candido E. Rivera, David J. Inwards, Ivana N. Micallef, Patrick B. Johnston, Luis F. Porrata, Stephen M. Ansell, Thomas M. Habermann, Thomas E. Witzig
Collection and assembly of data: Grzegorz S. Nowakowski, Betsy LaPlant, William R. Macon, Craig B. Reeder, Candido E. Rivera, David J. Inwards, Ivana N. Micallef, Patrick B. Johnston, Luis F. Porrata, Thomas E. Witzig
Data analysis and interpretation: Grzegorz S. Nowakowski, Betsy LaPlant, Craig B. Reeder, James Foran, Garth Nelson, Carrie Thompson, Thomas E. Witzig
Manuscript writing: All authors
Final approval of manuscript: All authors

REFERENCES

1. Coiffier B, Lepage E, Briere J, et al: CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. N Engl J Med 346:235-242, 2002
2. Habermann TM, Weller EA, Morrison VA, et al: Rituximab-CHOP versus CHOP alone or with maintenance rituximab in older patients with diffuse large B-cell lymphoma. J Clin Oncol 24:3121-3127, 2006
3. Sehn LH, Donaldson J, Chhanabhai M, et al: Introduction of combined CHOP plus rituximab therapy dramatically improved outcome of diffuse large B-cell lymphoma in British Columbia. J Clin Oncol 23:5027-5033, 2005
4. Pfreundschuh M, Trümper L, Osterborg A, et al: CHOP-like chemotherapy plus rituximab versus CHOP-like chemotherapy alone in young patients with good-prognosis diffuse large-B-cell lymphoma: A randomised controlled trial by the MabThera International Trial (MInT) Group. Lancet Oncol 7:379-391, 2006
5. Feugier P, Van Hoof A, Sebban C, et al: Long-term results of the R-CHOP study in the treatment of elderly patients with diffuse large B-cell lymphoma: A study by the Groupe d'Etude des Lymphomes de l'Adulte. J Clin Oncol 23:4117-4126, 2005
6. Maurer MJ, Ghesquières H, Jais JP, et al: Event-free survival at 24 months is a robust end point for disease-related outcome in diffuse large B-cell lymphoma treated with immunochemotherapy. J Clin Oncol 32:1066-1073, 2014
7. Klimo P, Connors JM: MACOP-B chemotherapy for the treatment of diffuse large-cell lymphoma. Ann Intern Med 102:596-602, 1985
8. Fisher RI, Gaynor ER, Dahlberg S, et al: Comparison of a standard regimen (CHOP) with three intensive chemotherapy regimens for advanced non-Hodgkin's lymphoma. N Engl J Med 328:1002-1006, 1993
9. Gordon LI, Harrington D, Andersen J, et al: Comparison of a second-generation combination chemotherapeutic regimen (m-BACOD) with a standard regimen (CHOP) for advanced diffuse non-Hodgkin's lymphoma. N Engl J Med 327:1342-1349, 1992
10. Cooper IA, Wolf MM, Robertson TI, et al: Randomized comparison of MACOP-B with CHOP in patients with intermediate-grade non-Hodgkin's lymphoma: The Australian and New Zealand Lymphoma Group. J Clin Oncol 12:769-778, 1994
11. Sertoli MR, Santini G, Chisesi T, et al: MACOP-B versus ProMACE-MOPP in the treatment of advanced diffuse non-Hodgkin's lymphoma: Results of a prospective randomized trial by the non-Hodgkin's Lymphoma Cooperative Study Group. J Clin Oncol 12:1366-1374, 1994
12. Alizadeh AA, Eisen MB, Davis RE, et al: Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 403:503-511, 2000
13. Hans CP, Weisenburger DD, Greiner TC, et al: Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood 103:275-282, 2004
14. Rosenwald A, Wright G, Chan WC, et al: The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. N Engl J Med 346:1937-1947, 2002
15. Wiernik PH, Lossos IS, Tuscano JM, et al: Lenalidomide monotherapy in relapsed or refractory aggressive non-Hodgkin's lymphoma. J Clin Oncol 26:4952-4957, 2008
16. Ivanov V, Tabouret E, Chuto G, et al: Rituximab-lenalidomide-dexamethasone induces complete and durable remission in relapsed refractory diffuse large B-cell non-Hodgkin lymphoma. Leuk Lymphoma 51:1758-1760, 2010
17. Witzig TE, Vose JM, Zinzani PL, et al: An International Phase II trial of single-agent lenalidomide for relapsed or refractory aggressive B-cell non-Hodgkin's lymphoma. Ann Oncol 22:1622-1627, 2011
18. Yang Y, Shaffer AL 3rd, Emre NC, et al: Exploiting synthetic lethality for the therapy of ABC diffuse large B cell lymphoma. Cancer Cell 21:723-737, 2012
19. Hernandez-Ilizaliturri FJ, Deeb G, Zinzani PL, et al: Higher response to lenalidomide in relapsed/refractory diffuse large B-cell lymphoma in nongerminal center B-cell-like than in germinal center B-cell-like phenotype. Cancer 117:5058-5066, 2011
20. Nowakowski GS, LaPlant B, Habermann TM, et al: Lenalidomide can be safely combined with R-CHOP (R2CHOP) in the initial chemotherapy for aggressive B-cell lymphomas: Phase I study. Leukemia 25:1877-1881, 2011
21. Cheson BD, Pfistner B, Juweid ME, et al: Revised response criteria for malignant lymphoma. J Clin Oncol 25:579-586, 2007
22. Zhang L, Qian Z, Cai Z, et al: Synergistic antitumor effects of lenalidomide and rituximab on mantle cell lymphoma in vitro and in vivo. Am J Hematol 84:553-559, 2009
23. Richardson PG, Schlossman RL, Weller E, et al: Immunomodulatory drug CC-5013 overcomes drug resistance and is well tolerated in patients with relapsed multiple myeloma. Blood 100:3063-3067, 2002
24. Lu L, Payvandi F, Wu L, et al: The anti-cancer drug lenalidomide inhibits angiogenesis and metastasis via multiple inhibitory effects on endothelial cell function in normoxic and hypoxic conditions. Microvasc Res 77:78-86, 2009
25. Wu L, Adams M, Carter T, et al: Lenalidomide enhances natural killer cell and monocyte-mediated antibody-dependent cellular cytotoxicity of rituximab-treated CD20+ tumor cells. Clin Cancer Res 14:4650-4657, 2008
26. Tilly H, Morschhauser F, Salles G, et al: Phase 1b study of lenalidomide in combination with rituximab-CHOP (R2-CHOP) in patients with B-cell lymphoma. Leukemia 27:252-255, 2013
27. Chiappella A, Tucci A, Castellino A, et al: 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. Haematologica 98:1732-1738, 2013
28. Vaidya R, Habermann TM, Donohue JH, et al: Bowel perforation in intestinal lymphoma: Incidence and clinical features. Ann Oncol 24:2439-2443, 2013
29. Czuczman MS: CHOP plus rituximab chemoimmunotherapy of indolent B-cell lymphoma. Semin Oncol 26:88-96, 1999
30. Rajkumar SV, Blood E: Lenalidomide and venous thrombosis in multiple myeloma. N Engl J Med 354:2079-2080, 2006
31. Zonder JA, Barlogie B, Durie BG, et al: Thrombotic complications in patients with newly diagnosed multiple myeloma treated with lenalidomide and dexamethasone: Benefit of aspirin prophylaxis. Blood 108:403, 2006
32. Caruso V, Di Castelnuovo A, Meschengieser S, et al: Thrombotic complications in adult patients with lymphoma: A meta-analysis of 29 independent cohorts including 18 018 patients and 1149 events. Blood 115:5322-5328, 2010

GLOSSARY TERMS

Bcl-6: the B-cell lymphoma-6 transcriptional repressor, containing conserved repressor and zinc finger motifs. Bcl-6 acts by recruiting co-repressor proteins.

CD10: initially identified as a common acute lymphoblastic leukemia antigen (and called CALLA). CD10 is a cell surface protein with zinc-binding metalloproteinase activity. It is expressed on the surface of neoplastic (eg, lymphoblastic, Burkitt's, and follicular germinal center leukemias) and normal (eg, early lymphoid progenitors, immature B and germinal B cells, T-cell precursors, and neutrophils) cells.

gene expression profiling: identifying the expression of a set of genes in a biologic sample (eg, blood, tissue) using microarray technology.