

Epratuzumab with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone chemotherapy in patients with previously untreated diffuse large B-cell lymphoma

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Approximately 60% of patients with diffuse large B-cell non-Hodgkin lymphoma (DLBCL) are curable with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) chemotherapy. Epratuzumab (E) is an unlabeled anti-CD22 monoclonal antibody with efficacy in relapsed DLBCL. This phase 2 trial tested the safety and efficacy of combining E with R-CHOP (ER-CHOP) in untreated DLBCL. A secondary aim was to assess the efficacy of interim positron

emission tomography (PET) to predict outcome in DLBCL. Standard R-CHOP with the addition of E 360 mg/m² intravenously was administered for 6 cycles. A total of 107 patients were enrolled in the study. Toxicity was similar to standard R-CHOP. Overall response rate in the 81 eligible patients was 96% (74% CR/CRu) by computed tomography scan and 88% by PET. By intention to treat analysis, at a median follow-up of 43 months, the event-free survival (EFS) and overall survival

(OS) at 3 years in all 107 patients were 70% and 80%, respectively. Interim PET was not associated with EFS or OS. Comparison with a cohort of 215 patients who were treated with R-CHOP showed an improved EFS in the ER-CHOP patients. ER-CHOP is well tolerated and results appear promising as a combination therapy. This study was registered at www.clinicaltrials.gov as #NCT00301821. (*Blood*. 2011;118(15):4053-4061)

Introduction

Diffuse large B-cell lymphoma (DLBCL) remains the most common type of non-Hodgkin lymphoma (NHL) in North America. The standard of care has evolved from combination chemotherapy with cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) to the addition of rituximab (R-CHOP). Various studies have demonstrated the benefit of adding rituximab to CHOP in both elderly and young patients.¹⁻⁴ Although R-CHOP improves both the overall response rate (ORR) and overall survival (OS), there remains room for improvement because ~20%-40% of patients still relapse after R-CHOP.¹⁻⁴

Epratuzumab is a humanized monoclonal IgG1 antibody directed against the B-cell specific antigen, CD22.^{5,6} CD22 is a 135-kDa transmembrane phosphoglycoprotein expressed by pre-B and mature, normal B cells. CD22 is a signaling molecule that plays a role in cellular adhesion, regulation of B-cell homing, and modulation of B-cell activation, and is internalized into the cell when bound by antibody. In vitro data have shown that pretreatment of B-cell lines with epratuzumab does not affect CD20 antigen expression; by contrast, pretreatment of the same cell lines with rituximab results in a slight increase in CD22 expression.⁵⁻⁷ The mechanism of action of epratuzumab is unknown; postulated mechanisms include antibody-dependent, cell-mediated cytotoxicity and apoptosis. In clinical studies, epratuzumab has shown

efficacy across various B-cell histologies. The phase 1/2 dose escalation trial by Leonard et al showed an ORR of 18% in heavily pretreated NHL patients and established the therapeutic dose of 360 mg/m² weekly × 4 doses.^{8,9} Combination antibody studies with epratuzumab and rituximab produced promising results with an ORR of 67% (complete response [CR], 50%) in DLBCL¹⁰ and in a European study the ORR was 47% (CR, 33%).¹¹

Because the addition of rituximab increased the efficacy of CHOP chemotherapy, we postulated that adding another antibody to target CD22 would further increase efficacy in DLBCL. A pilot (phase 1) study tested ER-CHOP in 15 patients with untreated DLBCL and determined the regimen was safe. The ORR was 87% (CR, 67%) with a 1-year progression-free survival (PFS) and OS of 93% and 100%, respectively.¹²

Positron emission tomography (PET) imaging is recommended at baseline and end of treatment for DLBCL patients.¹³ Retrospective studies have shown that the interim PET scans appear to be prognostic. In one study, PET scan after 2 cycles was highly predictive of outcome with the 2-year PFS for the PET-negative patients being 84% versus 0% for the PET-positive patients.¹⁴ However, recent reports have raised concerns about the false-positive rate of interim PET.^{15,16} In a report using PET scan in a risk-adapted study, 38 patients with a positive interim PET scan

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underwent repeat biopsy and 33 were negative for residual lymphoma.¹⁷ The PET Guided Therapy of Aggressive Non-Hodgkin Lymphomas Trial is a multicenter prospective trial in aggressive lymphoma exploring the prognostic value of interim PET. In this study, interim PET is performed after 2 cycles of therapy. The interval between chemotherapy and interim PET is more than 2 weeks, and granulocyte colony-stimulating factor is not permitted for the treatment cycle preceding interim PET. Quantitative standardized uptake value (SUV)-based assessment, rather than qualitative visual assessment, is applied. PET responsiveness is defined as reduction of the maximum SUV at interim PET by > 65% compared with the maximum pretreatment SUV. Using these criteria, treatment failure has been observed in 23% of the PET nonresponders versus only 8% of the PET responders.^{18,19}

Based on the promising results of the phase 1/pilot trial of ER-CHOP, we tested the regimen in a multicenter, phase 2 study through the North Central Cancer Treatment Group (NCCTG). The goals were to provide further data regarding the safety and efficacy of the regimen that would support a commitment to a large, definitive, phase 3 trial of ER-CHOP versus R-CHOP, and to provide important data on the role of functional imaging, both at interim PET scan and end of treatment in predicting event-free survival (EFS) in DLBCL.

Methods

Study design

This was a 1-arm open label phase 2 multicenter study through the NCCTG to assess the safety and efficacy of combination ER-CHOP in patients with newly diagnosed DLBCL. The objectives were to evaluate the efficacy as measured by 12-month EFS, PFS, OS, and response rates. In addition, functional CR rate (PET-negative) early (after 2 cycles, PET2) and at completion of therapy (after 6 cycles, PET6) was also studied.

After protocol completion, the results of the ER-CHOP-treated patients were compared with patients enrolled on a prospective, epidemiology study of the University of Iowa and Mayo Clinic SPORE Molecular Epidemiology Resource.^{20,21} The SPORE cohort consisted of all pathology confirmed DLBCL patients enrolled between September 2002 and February 2008, who met ER-CHOP trial eligibility criteria, were treated with R-CHOP, and were followed every 6 months for 3 years and annually thereafter.

Patients

Eligible patients were 18 years of age or older with previously untreated, CD20-positive DLBCL.²² Patients were required to have measurable disease, an absolute neutrophil count $\geq 1500/\mu\text{L}$, platelets $\geq 100\,000/\mu\text{L}$, bilirubin $\leq 2\text{ mg/dL}$, and creatinine $\leq 2\times$ the upper limit of normal. Patients were excluded if they had a prior diagnosis of NHL, evidence of indolent lymphoma on the biopsy or bone marrow, an Eastern Cooperative Oncology Group performance status of 3 or 4, active serious infection, poor cardiac function defined as an ejection fraction < 45%, central nervous system NHL, positive HIV status, or another primary malignancy within the preceding 5 years other than nonmelanomatous skin cancers, in situ carcinoma of the cervix, or treated prostate cancer with a stable prostate specific antigen, history of prior pelvic irradiation, and known hepatitis B or C infection. Staging and pretreatment assessment included clinical examination; computed tomography (CT) scans of the chest, abdomen, and pelvis; and a PET scan. Lumbar puncture was not required but was recommended in patients with known bone marrow or sinus involvement; patients who had positive cerebrospinal fluid cytology for lymphoma were excluded. A pregnancy test was required in women of child-bearing potential. The International Prognostic Index (IPI) was calculated based on age, serum lactate dehydrogenase, performance status, extranodal disease, and stage.²³ Extranodal sites of disease and stage were based on the CT scan results.

This study was conducted in accordance with the ethical guidelines mandated by the Declaration of Helsinki. All patients signed informed consent that was approved by the institutional review board at each participating site. In addition, all SPORE participants signed informed consent for inclusion in the prospective epidemiology study.

Epratuzumab (Immunomedics) 360 mg/m² was administered intravenously over 1 hour followed 30-60 minutes later by rituximab 375 mg/m² and CHOP chemotherapy (cyclophosphamide 750 mg/m² intravenously day 1, adriamycin 50 mg/m² intravenously day 1, vincristine 1.4 mg/m² [maximum 2 mg] intravenously day 1, and prednisone 100 mg/m² orally days 1-5). For cycle 1, rituximab could be divided into 2 days; if so, the second half of rituximab was given on day 2 followed by CHOP chemotherapy. For subsequent cycles, all therapy was administered on day 1. Premedication with acetaminophen and diphenhydramine was given before epratuzumab and repeated if necessary. Infusion-related side effects were treated per physician's discretion, including the use of corticosteroids. Routine use of antiemetics and granulocyte colony-stimulating factor were at physician's discretion and could be used prophylactically. Allopurinol was recommended during cycle 1 to prevent tumor lysis syndrome. Prophylactic intrathecal methotrexate was suggested but not required if there was involvement of sinuses, bone marrow, epidural space, or testicular sites.

Toxicity was graded according to National Cancer Institute Common Toxicity Criteria Version 3.0, with dose modifications based on adverse events. If patients experienced grade 4 neutropenia, granulocyte colony-stimulating factor was added to subsequent cycles. If hematologic toxicity was still present at day 1 of the next cycle (absolute neutrophil count $\leq 1500/\mu\text{L}$, or platelets $\leq 100\,000/\mu\text{L}$), then treatment was delayed by 1 week. If hematologic toxicity did not resolve after 3 weeks, the patient was removed from the study.

All biopsies were to be sent for central review (P.J.K.) within 7 days of registration, and treatment was initiated during central pathology review. Pathology criteria for the diagnosis of DLBCL was defined by the World Health Organization.²² Paraffin-embedded biopsies were stained for CD22 by immunohistochemistry using clones FPC1 (Novocastra) and TO15 (Dako North America). The lymphomas were judged to be CD22-positive if > 20% of the tumor cells were CD22-positive. Patients whose tumors did not meet the study criteria for DLBCL or whose lymphomas were CD22-negative were deemed ineligible. Epratuzumab was discontinued in these patients, and further therapy was at the discretion of the treating physician; however, most patients received R-CHOP.

Response assessment occurred after 2 and 6 cycles. Patients were observed for up to 5 years after treatment or until progression or death. CT scans were performed every 3 months year 1, every 4 months year 2, and every 6 months for years 3-5. PET scans were performed at baseline and after cycles 2 and 6. All PET scans were centrally reviewed without knowledge of patient's clinical course (G.A.W.) and were interpreted qualitatively as positive or negative. Fluorodeoxy-glucose (FDG) PET scan negativity was defined on the scan done after 2 cycles for therapy monitoring as no FDG uptake greater than the liver. On the FDG PET scan done after cycle 6 for restaging, the scan was read as negative if the tumor FDG uptake had returned to background.

Statistical methods

A 1-stage phase 2 design with an interim analysis based on a Fleming design was used.²⁴ The primary decision endpoint of the trial was the percentage of the first 67 eligible patients who were alive and event-free 12 months after enrollment to the study (EFS12). Secondary objectives included ORR, PFS, OS, functional CR, and safety. The design tested the null hypothesis that the EFS12 rate was $\leq 60\%$, based on the results of the Groupe d'Etude des Lymphomes de l'Adulte (GELA), Eastern Cooperative Oncology Group, and British Columbia Cancer Agency (BCCA) studies.¹⁻³ The statistical design had 91% power, with an α of 0.10, to declare the regimen was active if the true EFS12 rate was at least 75%.

EFS was defined as the time from study entry to the first event, with an event defined as tumor progression or relapse by CT scan, initiation of subsequent lymphoma therapy after ER-CHOP study therapy, or death from any cause. OS was defined as time from study entry to death from any cause. PFS was defined as the time from study entry to progression or death

Table 1. Baseline clinical characteristics

	Eligible (n = 81), no. (%)	Ineligible (n = 26), no. (%)	All (n = 107), no. (%)
Median age, y (range)	60 (21-82)	64 (29-81)	62 (21-82)
Age ≥ 60 y	42 (52)	16 (62)	58 (54)
Sex, female	35 (43)	13 (50)	48 (45)
PS 0 or 1	72 (89)	22 (85)	94 (88)
Stage III or IV	65 (80)	21 (81)	86 (80)
Elevated LDH	59 (73)	13 (50)	72 (67)
≥ 2 extranodal sites	22 (27)	10 (39)	32 (30)
IPI			
0-1	18 (22)	7 (27)	25 (23)
2	23 (28)	4 (15)	27 (25)
3	30 (37)	11 (42)	41 (38)
4-5	10 (12)	4 (15)	14 (13)
0-2	41 (51)	11 (42)	52 (49)
3-5	40 (49)	15 (58)	55 (51)
B symptoms	35 (43)	10 (39)	45 (42)
Bulky disease (> 10 cm)	16 (20)	3 (12)	19 (18)

because of lymphoma. Patients who had not experienced an event, death, or progression were censored at the last known follow-up. Response and progression were assessed by CT.²⁵ Functional response was defined as PET-negative.²⁶ Associations of categorical variables were compared using χ^2 and Fisher exact test. Comparisons of continuous variables were tested with the Wilcoxon rank-sum test. Survival, EFS, and progression curves were compared via Kaplan-Meier and the log-rank test²⁷; Cox proportional hazards models were used to assess the relationship between time-to-event endpoints and outcome.²⁸ All analyses were performed using SAS Version 9.1.3.

Results

Patient characteristics

Between February 2006 and August 2007, 107 patients with newly diagnosed DLBCL were enrolled. On-study patient characteristics are summarized in Table 1. Twenty-six (24%) patients were declared ineligible based on pathology review²⁵; 1 patient canceled before beginning treatment. The reasons for ineligibility resulting from pathology issues were: CD22-negative (11 patients), follicular lymphoma component in DLBCL sample,⁴ not DLBCL (3 grade 3 follicular lymphoma, 1 atypical Burkitt, 3 B-cell unclassifiable lymphoma), inadequate specimen (1), DLBCL with low grade in the marrow (1), and removal from study by treating physician because of c-myc translocation by FISH (1). Baseline characteristics between the eligible patients and ineligible patients were similar (Table 1). At the time of this analysis, the median follow-up was 43 months (range, 7-58 months); 31 patients (29%) had an event and 22 patients (21%) had died.

Safety

In general, ER-CHOP treatment was well tolerated. A total of 106 patients received at least 1 cycle, and 505 treatment cycles were analyzed for toxicity. Seventy-two of the 81 eligible patients (89%) received all 6 cycles of therapy on study. Three patients went off study for progression; other reasons included adverse event (1), patient refusal (2), death on study (1), and clinical deterioration (2). Hematologic toxicity was monitored with weekly complete blood count. The rates of grades 3 or 4 hematologic toxicity were 14% for anemia, 85% for neutropenia, and 14% for thrombocytopenia. Despite the substantial incidence of neutropenia, febrile neutropenia was observed in 16% (17 of 106) of cases. Other

toxicities (Table 2) were typical for CHOP-based therapy. Overall, 45 patients (42%) experienced at least 1 grade 3 or higher toxicity, and 13 patients (12%) experienced grade 4 or 5 treatment-related toxicity. Three patients died on study; 1 patient died 12 days after day 1 cycle 1 because of exacerbation of chronic obstructive pulmonary disease; 1 patient died of pneumonia during cycle 6, and 1 patient died of ventricular dysfunction 1 month after completing therapy.

Treatment cycles were able to be delivered on schedule in 72% (76 of 106) of cases and 93% (469 of 505) of treatment cycles. The most common single reason for delay was hematologic toxicity.¹¹ Dose reductions were required in 25% (26 of 106) of patients and in 9% (43 of 505) of treatment cycles primarily for cyclophosphamide and doxorubicin (20 cycles) and prednisone (10 cycles).

Response and outcome assessment

The study met the prespecified study design criteria for efficacy at the interim and final analyses. Survival results are presented as intention to treat for all 107 patients. The OS, PFS, and EFS at 36 months were 80%, 76%, and 70% in all patients and 79%, 75%, and 69% eligible patients, respectively (Figure 1). By IPI, 25 (23%) patients were low-risk (IPI 0-1), 68 (64%) patients were intermediate-risk, and 14 (13%) were high-risk (IPI 4-5). The outcomes by IPI subgroup are shown in Figure 2.

CT and PET results are available for the eligible patients as per study design. All PET scans were centrally reviewed (Table 3). The ORR was 96% (74% CR/CRu; 22% partial response [PR]) using standard response assessment by CT (Table 3). Functional CR, as defined by PET negativity, was assessed after 2 cycles and after completion of treatment. PET scans were available in 94% (76 of 81) of eligible patients. Early PET (PET2) was negative in 78% (54 of 69) and PET after completion of therapy (PET6) was negative in 88% (61 of 69). Overall, 87% of patients (67 of 77) achieved PET-negative status during the study. PET2 negativity was not associated with a statistically significant improvement in EFS ($P = 0.31$) or OS ($P = .24$). However, PET6 negativity was associated with a statistically significant improvement in EFS ($P = .02$) and OS ($P = .002$). At 3 years, the EFS if PET6-negative was 78% compared with 50% if still PET6-positive; OS was 90% for PET6-negative compared with 50% for PET6-positive (Figure 3). Overall, 3-year survival in the 67 patients achieving a PET2- or PET6-negative status was 73% compared with 44% in the patients who did not achieve a PET-negative status ($P = .02$). Of the 15 patients that were PET2-positive, 8 became PET6-negative, of which 5 remain in CR. Of the 54 patients that were PET2-negative,

Table 2. Most common (> 10%) adverse events without regard to grade and attribution

Toxicity	Count	%
Neutropenia	99	93.4
Thrombocytopenia	71	67.0
Neurosensory	48	45.3
Alopecia	40	37.7
Fatigue	38	35.8
Anemia	35	33.0
Vomiting	31	29.2
Hyperglycemia	18	17.0
Febrile neutropenia	17	16.0
Cytokine release	15	14.2
Nausea	13	12.3
Dyspnea	11	10.4

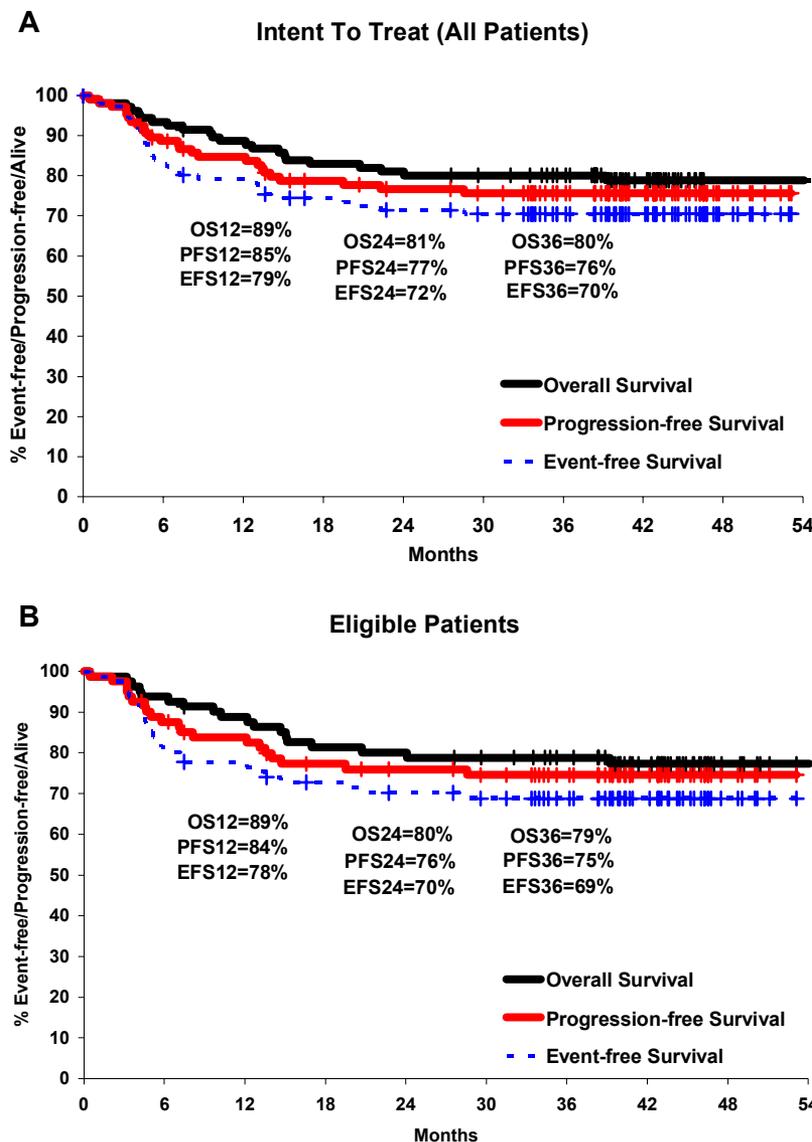


Figure 1. Time-to-event curves for EFS, PFS, and OS. (A) Intention-to-treat patients. (B) Eligible patients.

48 (89%) completed treatment in PET CR; 3-year EFS and OS were 79% and 92%, respectively, in these 48 patients.

Comparison with SPORE cohort

The cohort of R-CHOP-treated patients in the SPORE had similar baseline characteristics to the ER-CHOP trial patients, with the exception of ER-CHOP patients being higher stage (Table 4). After adjusting for IPI, the ER-CHOP patients had improved EFS (hazard ratio = 0.65; 95% confidence interval, 0.43-0.98, $P = .04$; Figure 4). Improvement in OS was similar (hazard ratio = 0.68; 95% confidence interval, 0.42-1.11, $P = .12$), although the association was not statistically significant because of a fewer number of events in the OS analysis (Figure 4). The improved outcome of patients treated with ER-CHOP compared with the patients treated with R-CHOP (SPORE cohort) was similar across IPI groups; however, the statistical power was inadequate to make definitive conclusions (data not shown).

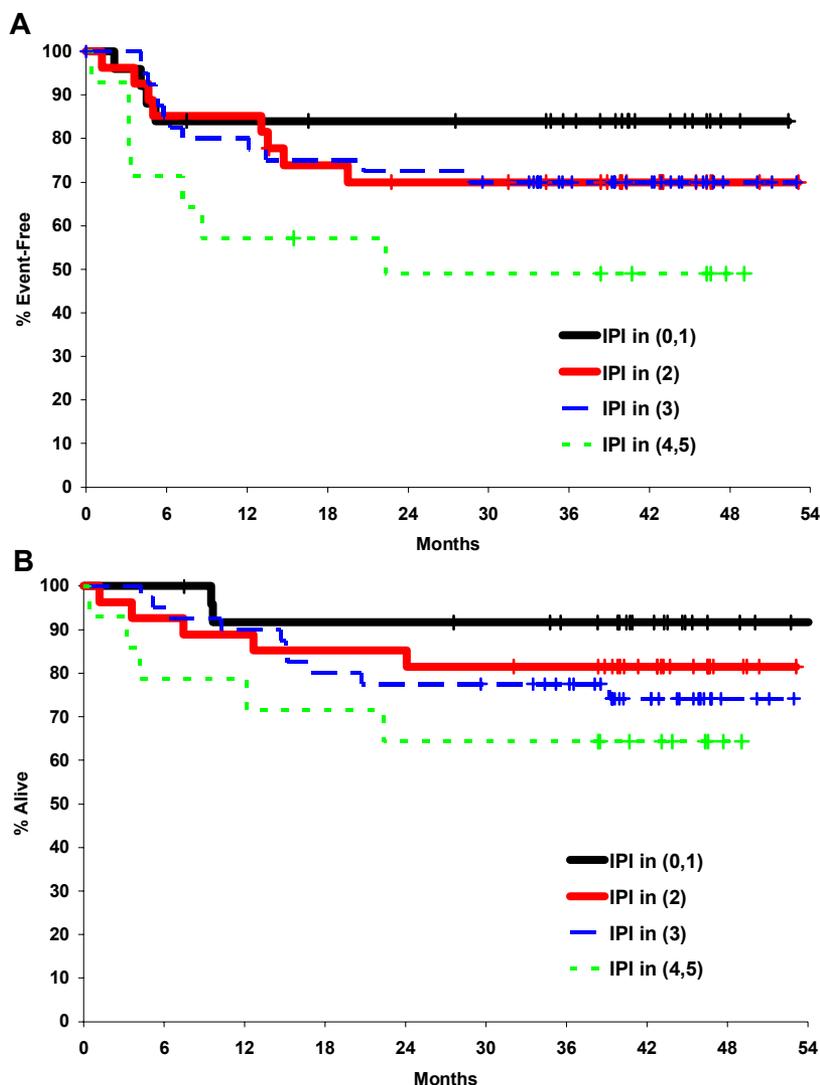
Discussion

The results of this phase 2 study reported herein demonstrate an ORR of 96% to ER-CHOP, a functional CR rate (PET-negative) of

88%, 3-year EFS of 70%, and OS of 80%. The strengths of the study include the large sample size from a multicenter cooperative group clinical trial and mature follow-up of trial participants. Although the limitations of the study include the lack of a randomized arm of patients treated with standard R-CHOP therapy and the ineligibility of CD22-negative patients, we attempt to address these issues by intention-to-treat analysis and comparison with other R-CHOP patient datasets, including a prospectively enrolled cohort of DLBCL patients from our SPORE who fulfilled the ER-CHOP trial eligibility criteria. Therapy with R-CHOP delivered every 21 days is the standard of care for patients with untreated DLBCL with adequate cardiac function that permits anthracycline use. Improving R-CHOP for DLBCL is very important. Salvage therapy for those that relapse, especially patients older than 70 years and refractory/early relapse patients, is toxic and is associated with very poor outcomes.²⁹ Recently, GELA reported 10-year long-term follow-up of the original R-CHOP versus CHOP study patients. The median OS after progression was 0.6 and 0.7 months for the CHOP and R-CHOP arms.³⁰

Various studies have tried to improve the results of treating DLBCL by either adding chemotherapeutic agents, such as etoposide (cyclophosphamide, doxorubicin, vincristine, etoposide, prednisone

Figure 2. Time-to-event curve by IPI status. (A) EFS. (B) OS.



[CHOEP]), by increasing dose density (administering chemotherapy every 14 days) or using infusional regimens dose adjusted (DA)-EPOCH (etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin).³¹⁻³³ Phase 3 studies evaluating R-CHOP-14 versus R-CHOP-21 and the infusional chemotherapy regimen of dose-

adjusted EPOCH-R versus R-CHOP-21 are ongoing.³⁴⁻³⁶ Cunningham et al reported the preliminary results of the United Kingdom study comparing 8 cycles of R-CHOP-21 versus 6 cycles of R-CHOP-14 plus 2 additional cycles of rituximab.³⁴ The ORRs were equivalent at 88% for R-CHOP-21 versus 91% for R-CHOP-14. There was also no advantage to R-CHOP14 versus R-CHOP21 in the French study.³⁵ We await long-term follow-up to determine whether there will be a difference in relapse and/or survival.

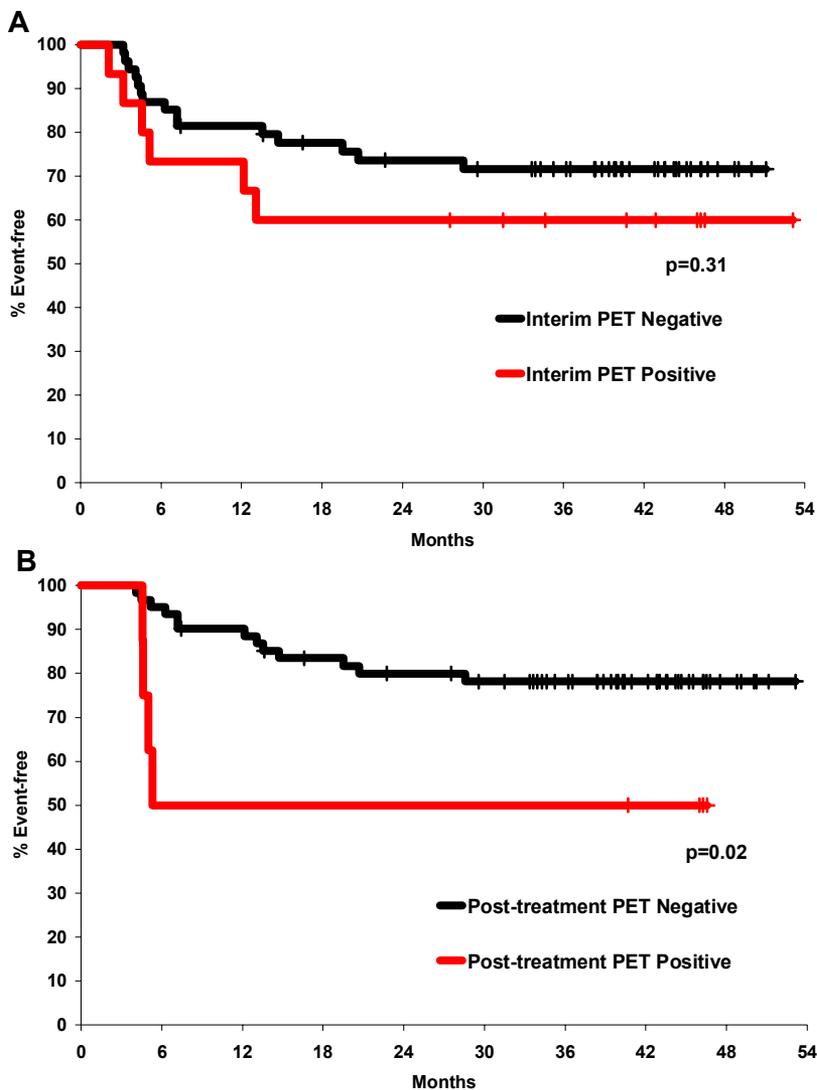
In this study, we used the approach of targeting another cell surface antigen (CD22) with epratuzumab combined with standard R-CHOP. The CD22 antigen is an attractive additional target to CD20 because of different mechanisms of action and possible additive or synergistic effects.^{10,11} This dual-antibody targeting may help to overcome antibody resistance and therefore improve responses and ultimately survival without complicating the R-CHOP schedule or adding significant toxicity.

The results with ER-CHOP suggest an improvement in OS, EFS, and PFS compared with the initial randomized clinical trials using R-CHOP. However, those studies were in older patients.^{1,2} The population-based BCCA data, which are in all ages, have an OS and PFS at 4 years of ~70% compared with 79% and 76%, respectively, in our study of ER-CHOP.³ We also compared the

Table 3. Response assessment

	After cycle 2 scan, no. (%)	After cycle 6 scan, no. (%)
CT (n = 81)		
CR	12 (15)	27 (33)
CRu	9 (11)	18 (22)
PR	49 (60)	32 (40)
SD	8 (10)	2 (2)
NA	3 (4)	2 (2)
CR/Cru	21 (26)	45 (55)
ORR	70 (86)	77 (95)
PET		
PET-positive	15 (22)	8 (11)
PET-negative	54 (78)	61 (88)*

*Patients who were PET-negative after 2 cycles and completed treatment and did not have a posttreatment PET scan were considered PET-negative after treatment if the posttreatment CT scan did not show progression.



ER-CHOP trial results with patients prospectively enrolled to the University of Iowa and Mayo Clinic Lymphoma SPORE Molecular

Table 4. Baseline clinical characteristics of ER-CHOP patients and SPORE patients

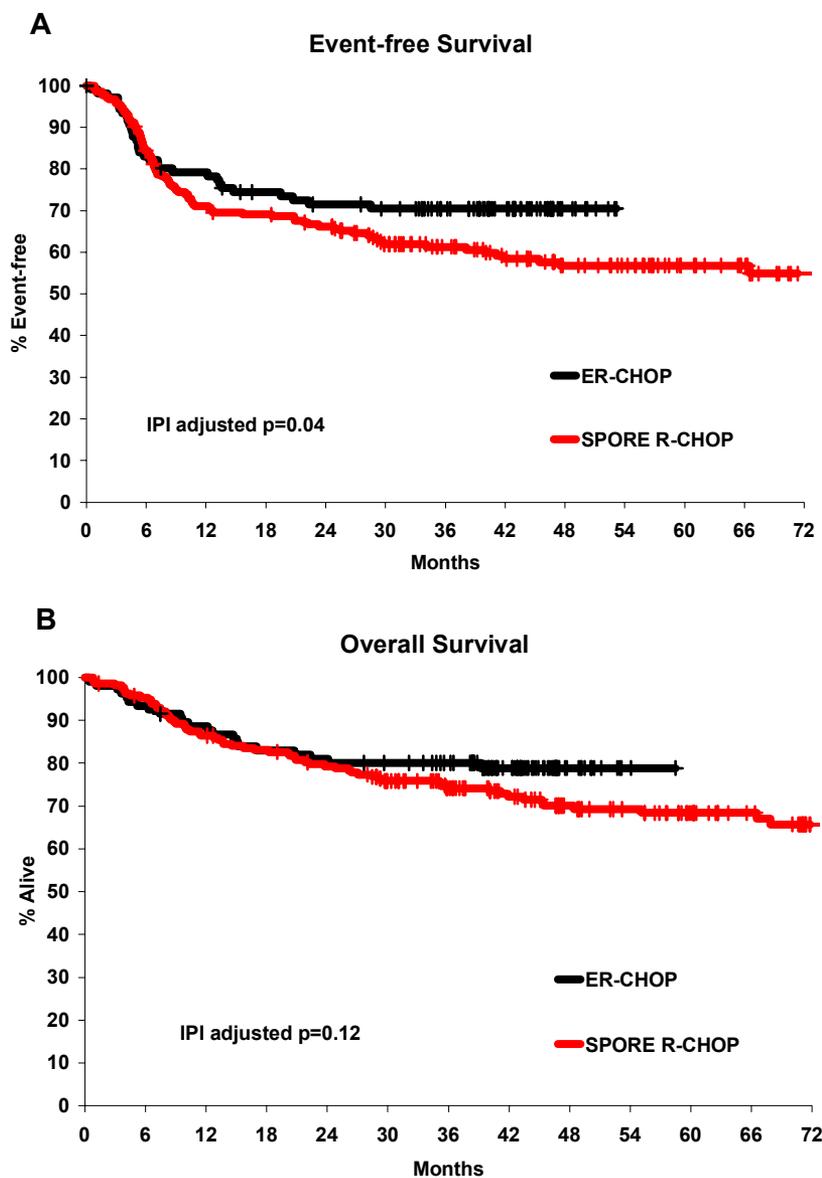
	ER-CHOP (n = 107), no. (%)	SPORE R-CHOP (n = 215), no. (%)	P
Median age, y (range)	62 (21-82)	62 (21-92)	
Age ≥ 60 y	58 (54)	121 (56)	.72
Sex, female	48 (45)	100 (47)	.78
PS 0 or 1	94 (88)	179 (83)	.28
Stage III or IV	86 (80)	144 (67)	.01
Elevated LDH	72 (67)	118 (58)	.13
≥ 2 extranodal sites	32 (30)	55 (26)	.45
IPI			
0-1	25 (23)	61 (28)	.33
2	27 (25)	63 (29)	
3	41 (38)	61 (28)	
4-5	14 (13)	30 (14)	
0-2	52 (49)	124 (58)	
3-5	55 (51)	91 (42)	
B symptoms	45 (42)	64 (30)	.12
Bulky disease (> 10 cm)	19 (18)	33 (15)	.62

Epidemiology Resource.^{20,21} Although the survival curves are similar for the first 2 years, the curves diverge at 2 years, favoring patients treated with ER-CHOP. This suggests that epratuzumab may decrease late relapses.

The ER-CHOP results evaluated by IPI status also show an improvement, especially in the high-risk patients. Four-year OS in the high-intermediate and high-risk IPI³⁻⁵ was 55% in the BCCA cohort and 58% in the SPORE cohort compared with 72% at 4 years with ER-CHOP. Improvement was similar for 4-year PFS (53% in BCCA vs 71% ER-CHOP) and EFS (41% in SPORE vs 65% with ER-CHOP) as well. At a median follow-up of nearly 4 years, one patient has progressed after 24 months after initiation of ER-CHOP on our study, suggesting that there is a plateau. In the GELA 10-year follow-up analysis, 87% of relapses occurred during the first 3 years of follow-up.³⁰ Therefore, a large number of late relapses would not be expected for this analysis.

Approximately 85% of patients with DLBCL are CD22-positive.⁷ We defined CD22 positivity by immunohistochemistry as > 20% positive staining on tumor cells. This cut-off was chosen because it is a cut-off commonly used in hematopathology to determine antigen expression by immunohistochemistry. We found that in nearly all cases the tumor cells were either positive or

Figure 4. Comparison of ER-CHOP intention-to-treat results with R-CHOP treated patients from the University of Iowa/Mayo Clinic SPORE Molecular Epidemiology Resource. (A) EFS. (B) OS.



negative by immunohistochemistry. By gene profiling, CD22 parallels CD20; thus, the CD22-negative cases by immunohistochemistry in our study may represent staining below the level of detection of immunohistochemistry. There have been no reports of CD22 as a prognostic marker; therefore, by removing CD22-negative patients in this study, it is unlikely that a poor prognostic group was removed. Interestingly, by intention-to-treat analysis, there was no difference in survival outcomes between the eligible and ineligible patients. In the previous pilot study, 11 of 15 patients had CD22 status assessed. Of these 11, all were CD22-positive, 2 of whom were weakly positive. Both of these weakly positive patients achieved a CR and remain alive without disease. In the single-agent radioimmunotherapy studies using ^{90}Y -epratuzumab tetraxetan in 64 patients with relapsed/refractory NHL, 98% of patients demonstrated tumor targeting on ^{111}In imaging.³⁷

In this study, we also found that outside of clear-cut progression, using a positive PET2 to change therapy is not supported. Fifteen patients were PET2-positive, of whom 8 became PET6-negative and 5 remain progression-free. Quantitative SUV-based

interpretation showing PET responsiveness may, however, be more important than qualitative interim PET assessment where a scan is interpreted as positive or negative. In a post hoc analysis, the interim positive PET2 scans were reviewed as a quantitative SUV-based assessment. As per the PET Guided Therapy of Aggressive Non-Hodgkin Lymphomas Trial, PET nonresponse was defined as active disease by visual criteria and a maximum SUV reduction of $\leq 65\%$.¹⁸ Fifteen patients had an interim PET2 scan that was originally read as positive. Twelve of these were available for review; in these 12 patients, 9 were classified as PET responders and 3 nonresponders. Of the 3 nonresponders, 2 progressed early and 1 continues in remission (> 2 years).

PET negativity at completion of therapy (PET6) was associated with an improved EFS and OS. The EFS and OS at 3 years in patients who are PET6-negative are 78% and 90%, respectively. Of the 8 patients who were PET6-positive, 4 remain alive without progression. In contrast, of the 61 patients who were PET6-negative, 6 (10%) have relapsed. Thus, negative PET6 serves as a valuable surrogate marker for EFS and a valid study endpoint for future clinical trials.

In conclusion, the addition of epratuzumab may increase the efficacy of R-CHOP without added toxicity. This benefit is especially evident in the high-intermediate and high-risk IPI patients. A randomized phase 3 trial of R-CHOP versus ER-CHOP is required to definitively demonstrate that dual-antibody targeting in combination with CHOP results in improved outcomes.

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Authorship

Contribution: I.N.M.M. was the principal investigator of the study, designed the study, analyzed the data, and wrote the manuscript; M.J.M. was the biostatistician; G.A.W. was the central reviewer for the PET scans; D.A.N. provided patients and reviewed the manuscript; P.J.K. reviewed pathology specimens and reviewed the manuscript; M.W.C., D.G.P., G.S.S., T.M.H., and B.K.L. provided patients and reviewed the manuscript; and T.E.W. assisted in clinical trial development, supplied patients, and participated in writing the manuscript.

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A complete list of participating institutions appears in the supplemental Appendix, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article.

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Epratuzumab with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone chemotherapy in patients with previously untreated diffuse large B-cell lymphoma

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