R-CHOP with or without bevacizumab in patients with previously untreated diffuse large B-cell lymphoma: final MAIN study outcomes

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ABSTRACT

Vascular endothelial growth factor is involved in lymphoma growth, suggesting a potential role for anti-vascular endothelial growth factor therapies in hematologic malignancies. In this phase III study, patients with CD20-positive diffuse large B-cell lymphoma were randomized to rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone plus either placebo (R-CHOP) or bevacizumab (RA-CHOP). Treatment was administered every 21 (8 cycles) or 14 days (6 cycles plus 2 rituximab cycles) as per institutional practice. An early analysis of risk/benefit by the Data and Safety Monitoring Board showed that RA-CHOP increased cardiotoxicity without prolonging progression-free survival compared with R-CHOP, and the trial was stopped early. The study protocol was amended to allow for 12 additional months of follow up to evaluate safety. With 787 patients enrolled, median follow up was 23.7 and 23.6 months for R-CHOP and RA-CHOP, respectively. Median progression-free survival for R-CHOP and RA CHOP was 42.9 and 40.2 months, respectively (hazard ratio=1.09; *P*=0.49). The proportion of deaths was identical for R-CHOP (83 of 387, 21%) and RA-CHOP (82 of 390, 21%). Relative to R-CHOP, RA-CHOP had a higher rate of left ventricular ejection fraction perturbation (18% *vs.* 8%; odds ratio=2.51; 95% confidence interval (CI): 1.60-3.93) and congestive heart failure (16% *vs.* 7%; odds ratio=2.79; 95%CI: 1.72-4.54). Bevacizumab added to R-CHOP increased cardiac events, without increasing efficacy, arguing against further evaluation of RA-CHOP in patients with diffuse large B-cell lymphoma. The MAIN study is registered at *clinicaltrials.gov identifier:00486759*.

Introduction

Angiogenesis in general and vascular endothelial growth factor (VEGF) in particular are involved in the development, growth, and progression of a range of non-Hodgkin lymphoma (NHL) histological subtypes,[1-5] including diffuse large B-cell lymphoma (DLBCL).[6-8] High serum levels of VEGF and elevated expression of VEGF in tissue biopsies are associated with higher tumor burden,[9] microvessel density,[3,6] and inferior overall survival (OS).[4,7,8,10] *In vitro* and *in vivo* pre-clinical studies of DLBCL have shown that treatments targeting VEGF or its receptors reduce tumor growth by increasing apoptosis and decreasing vascularization.[5] The potential therapeutic role of anti-VEGF therapies in NHL has also been evaluated in preliminary clinical studies of bevacizumab. Bevacizumab is an anti-VEGF monoclonal antibody that has been studied extensively in a range of solid tumors. Bevacizumab has been shown to have negligible or modest antitumor activity as a single agent,[11] but when combined with standard chemotherapy, it confers substantial improvements in progression-free survival (PFS) and OS in patients with non-squamous non-small cell lung cancer[12,13] and metastatic colorectal cancer.[11,14] In a Southwest Oncology Group (SWOG) phase II study of 52 patients with relapsed DLBCL or mantle cell lymphoma (MCL), bevacizumab monotherapy was well tolerated and associated with a 6-month PFS rate of 16% and a median duration of response or stable disease of 5.2 months. Although the objective response rate (ORR) was low (2%, one partial response (PR) in a patient with DLBCL), single-agent activity was not anticipated given its mechanism of action; combination therapy trials were thus deemed appropriate.[15] The anti-CD20 monoclonal antibody rituximab used in combination with cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) chemotherapy is standard treatment for DLBCL.[16-22] However, there is a need to further improve treatment outcomes, as the 2-year PFS rate of a large population-based analysis of R-CHOP was only 69%.[23] In a second SWOG study, the feasibility and safety of adding bevacizumab to R-CHOP (RA-CHOP) was explored in 13 patients with newly diagnosed DLBCL. An ORR of 85% and a 12-month PFS rate of 77% were observed. RA-CHOP was well tolerated, with no reported episodes of grade 3-4 heart failure or hemorrhage.[24]

The phase III, randomized, placebo-controlled, rituximab plus bevacizumab in aggressive NHL (MAIN) study was undertaken (*clinicaltrials.gov identifier:00486759*), to compare PFS with R-CHOP plus placebo (R-CHOP) with RA-CHOP in patients with previously untreated CD20-positive DLBCL. However, after the study's Data and Safety Monitoring Board (DSMB) noted an increased risk of cardiac events without improvement in efficacy for RA-CHOP over R-CHOP, treatment with bevacizumab was discontinued. The protocol was then modified, and the primary end point was changed from PFS to safety follow up. Final efficacy and safety data from the MAIN study are reported here.

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Methods

The MAIN study randomized (1:1) patients aged 18 years or over with newly-diagnosed CD20-positive DLBCL to RA-CHOP or R-CHOP in six 14-day cycles (plus two extra doses of rituximab) or eight 21-day cycles as per standard practice at individual centers (Figure 1A).

Adverse events (AEs) were monitored by an independent DSMB. The DSMB noted a trend for increased cardiotoxicity among patients randomized to RA-CHOP *versus* R-CHOP at its December 2009 meeting, when 609 patients had been enrolled. Patients and investigators were informed of the potentially increased risk of cardiac events associated with RA-CHOP. In May 2010, with 770 randomized patients and efficacy data available from 720 of them, the DSMB concluded that adding bevacizumab to R-CHOP would be unlikely to improve efficacy; the increased risk of cardiotoxicity persisted. On May 31, 2010, the sponsor terminated enrollment and discontinued treatment with bevacizumab/placebo. Patients continued treatment with R-CHOP. Safety became the revised primary end point. Patients were followed for up to 12 months after the last patient received last chemotherapy dose.

Interim response was assessed after three cycles of R(A)-CHOP-14 or four cycles of R(A)-CHOP-21 as per Revised Criteria for Malignant Lymphoma (Cheson criteria).[25] PET scans were not mandatory for response assessment as they were not standard when the study was designed, and consequently not available at all study centers. Patients with progressive disease or stable disease discontinued study medication and immediately entered a follow-up phase examining disease progression and survival. Patients with complete response (CR) or PR received three additional cycles of R(A) CHOP-

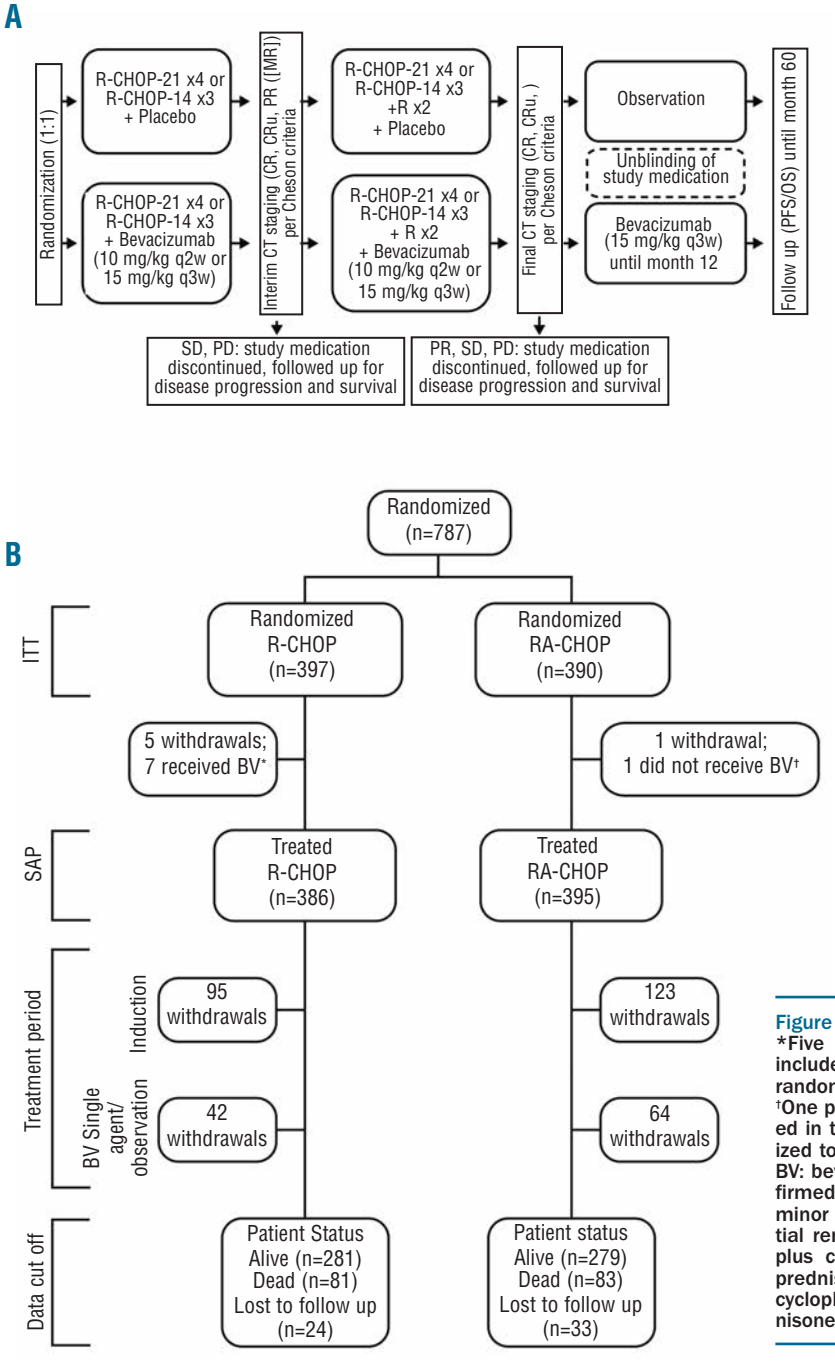

Figure 1. (A) Study design and (B) patient disposition. *Five patients did not receive study drug and were included in the ITT, but not in the SAP. Seven patients randomized to R-CHOP received bevacizumab in error. †One patient did not receive study drug and was included in the ITT, but not in the SAP. One patient randomized to RA-CHOP did not receive bevacizumab in error. BV: bevacizumab; CR: complete response; CRu: unconfirmed complete response; ITT: intent to treat; MR: minor response; PFS: progression-free survival; PR: partial remission; OS: overall survival; R-CHOP: rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone; RA-CHOP: bevacizumab plus rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone; SAP: safety analysis population.

14 or four cycles of R(A)-CHOP-21, with another assessment performed at induction end. It was not anticipated that the difference in time of re-staging between 14- and 21-day groups would affect the results of the study. Although a more straightforward interpretation of data may have been possible with the choice of either a 14- or 21-day cycle for this study, the current design allowed centers to participate using their usual therapy arrangements.

AEs (NCI-CTCAE, v. 3.0) were recorded at each scheduled visit until three months post treatment. AEs of special interest were documented until six months post treatment. Left ventricular ejection fraction (LVEF) and congestive heart failure (CHF) events and related serious AEs (SAEs) were monitored indefinitely. Strict cardiac monitoring was protocol-specified, with LVEF measured at baseline via 2D-echocardiogram or multigated acquisition scan, after cycles 4 and 8, and at month 12 (using the same assessment method as the base-line assessment). Patients with an LVEF or CHF event continued follow up at 3-monthly intervals until resolution. The severity of CHF events was defined as per NCI-CTAE v. 3.0, and LVEF events were defined as a decline from baseline of 20% or more in LVEF or a decline from baseline of ≥10% or more to an LVEF value of less than 50% baseline.

Efficacy analyses were performed on the intent-to-treat population, which was composed of all randomized patients regardless of treatment administration. The safety population was composed of patients who received at least one dose of study medication and had at least one safety follow-up visit. The distributions of PFS and OS over time were compared between RA-CHOP and R-CHOP using the two-sided log rank test (alpha=0.05). An estimate of the hazard ratio (HR) was obtained from Cox regression analyses. Kaplan-Meier estimates of the median were calculated. The risk of an LVEF or CHF event was compared between treatments using logistic regression, with an estimate of odds ratio. Logistic regression for CHF events was performed in various subgroups, with multivariate logistic regression used to further assess the impact of treatment and base-line characteristics on CHF event risk. Time to first CHF event and cumulative dose of bevacizumab/doxorubicin at first CHF event were analyzed using Kaplan-Meier methodology.

The study was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice (GCP). The protocol and all accompanying material provided to patients were reviewed and approved by institutional ethics committees (IEC) or institutional review boards (IRB) prior to the start of the study. Protocol amendments were also approved by the IECs/IRBs.

Results

Efficacy

From July 26, 2007 to May 31, 2010, 787 patients had been randomized (R-CHOP, n=397; RA CHOP, n=390) (Figure 1B). Demographics, base-line disease characteristics, and treatment scheduling were well balanced between treatment arms (Table 1). The median durations of follow up for the R-CHOP and RA-CHOP arms were 23.7 and 23.6 months, respectively. A total of 31% and 32% of patients experienced a PFS event in the R-CHOP and RA-CHOP arms, respectively. Median PFS for R-CHOP was 42.9 *versus* 40.2 months for RA-CHOP (HR=1.09; 95%CI: 0.85-1.40; P =0.49) (Figure 2A). Median OS had not been reached in either treatment arm by the end of follow up, when 165 patients had died (R-CHOP, 21%; RA-CHOP, 21%). No significant difference in the HR for death was found between treatments (HR=1.03; 95%CI:

Table 1. Demographics and base-line disease characteristics of the ITT population.

	R-CHOP (n=397)	RA-CHOP (n=390)
Female sex, n (%)	193 (49)	207 (53)
Median age, years (range)	61 (18-82)	61 (19-89)
<65 years, n (%)	241 (61)	247 (63)
≥65 years, n (%)	156 (39)	143 (37)
Geographical region, n (%)	212 (53)	209 (54)
Eastern and Western Europe	87 (22)	85 (22)
South, Central, and North America	98 (25)	96 (25)
Other		
ECOG PS, n/N (%)		
0–1	321/395 (81)	308/389 (79)
2–3	74/395 (19)	81/389 (21)
IPI risk factors, n/N (%)		
0-1	65/397 (16)	48/389 (12)
2	162/397 (41)	177/389 (46)
3–5	170/397 (43)	164/389 (42)
Bulky disease (≥7.5 cm), n (%)	198 (50)	195 (50)
Median LVEF (range)	65 (47-90)	65 (48-88)
Elevated LDH (>ULN), n (%)	259 (65)	252 (65)
Treatment schedule, n (%)		
R-CHOP-14	81 (20)	80 (21)
R-CHOP-21	316 (80)	310 (80)

ECOG PS: Eastern Cooperative Oncology Group performance status; IPI: international prognostic index; ITT: intent to treat; LDH: lactate dehydrogenase; LVEF: left ventricular ejection fraction; R-CHOP: rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone; RA-CHOP: bevacizumab plus rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone; ULN: upper limit of normal.

Table 2. Summary of the most common adverse events.

	R-CHOP (n=386)	RA-CHOP (n=395)
Patients with any AE, n (%)*	362 (94)	387 (98)
Nausea	106 (28)	108 (27)
Neutropenia	125 (32)	93 (24)
Diarrhea	84 (22)	99 (25)
Patients who discontinued any treatment due to an AE, n (%)	47 (12)	100 (25)
Left ventricular dysfunction	5	19 (5)
Ejection fraction decrease	5	8
Febrile neutropenia	2	6
Patients with AE of special interest to bevacizumab (any grade), n (%)	96 (25)	187 (47)
Bleeding	31 (8)	77 (19)
CHF†	22 (6)	64 (16)
Hypertension	14 (4)	64 (16)
Venous thrombosis	19 (5)	17 (4)
Cardiac disorders (excluding CHF and ATEs)	11 (3)	13 (3)
Gastrointestinal perforation	3	11 (3)
Patients with SAE, n (%)	173 (45)	224 (57)
Febrile neutropenia	48 (12)	63 (16)
Pneumonia	16 (4)	22 (6)
Pyrexia	15 (4)	14 (4)

**Percentages have not been calculated for patient values <10. †Includes CHF events that were reported up to the standard cut off for reporting AEs of special interest (Day 183). AE: adverse event; ATE: arterial thrombotic event; CHF: congestive heart failure; R-CHOP: rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone; RA-CHOP: bevacizumab plus rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone; SAE: serious adverse event.*

0.76-1.40; *P*=0.84) (Figure 2B). The proportion of responders at final staging was higher for R-CHOP than RA-CHOP (71% *vs.* 63%), with 54% and 47% of patients achieving CR/unconfirmed complete response in the R-CHOP and RA-CHOP arms, respectively. The absolute difference in overall response rate between treatments was –7.5% (95%CI: –14.2% to –0.8%).

Safety

Of the 787 patients randomized, 781 (99%) received at least one cycle of study medication (R-CHOP, n=386; RA CHOP, n=395). In the RA-CHOP arm, the median total cumulative dose of bevacizumab was 113.3 mg/kg (range 10.0-313.4 mg/kg). Similar proportions of patients experienced at least one AE in the 2 treatment arms (94%, R-CHOP; 98%, RA-CHOP), with the most common events being neutropenia, nausea, and diarrhea (Table 2). Respiratory, thoracic, and mediastinal disorders (31% *vs.* 40%); vascular disorders (17% *vs.* 25%); and cardiac disorders (12% *vs.* 19%) were observed less frequently in patients randomized to R-CHOP than RA-CHOP. The majority of patients experienced AEs of grade 2 intensity (R-CHOP, 80%; RA-CHOP, 84%), but numerically more RA-CHOP-treated patients experienced grade 3/4 (58% *vs.* 55%) or grade 5 AEs (8% *vs.* 5%). The percentage of patients discontinuing bevacizumab/placebo due to an AE was greater for RA-CHOP than for R-CHOP (23% vs. 11%), and constituted the majority of AE-related discontinuations overall (Table 2). A total of 695 SAEs were reported in 397 patients (R-CHOP, 45%; RA-CHOP, 57%). The only SAEs with a 2% or higher incidence in the RA-CHOP than R-CHOP arm were febrile neutropenia (R-CHOP, 12%; RA-CHOP, 16%) and left ventricular dys-

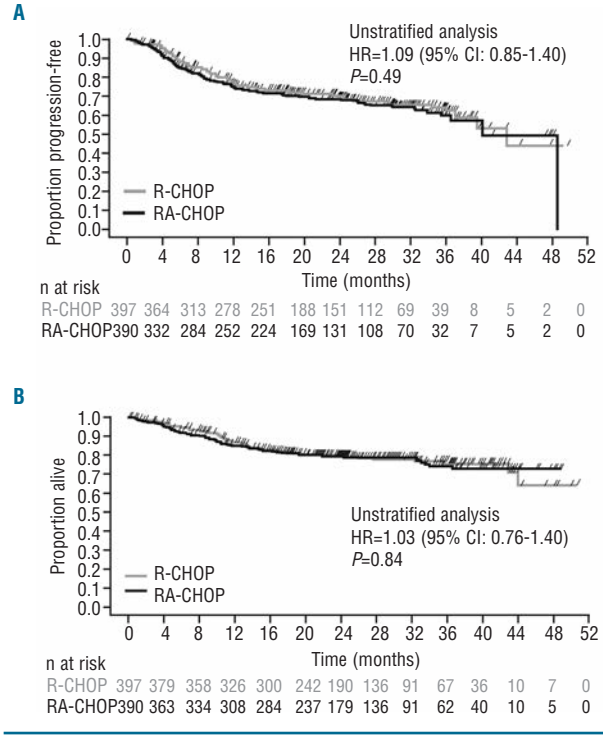

Figure 2. Kaplan-Meier analysis of (A) PFS and (B) OS by treatment arm. CI: confidence interval; HR: hazard ratio; PFS: progression-free survival; OS: overall survival; R-CHOP: rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone; RA-CHOP: bevacizumab plus rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone.

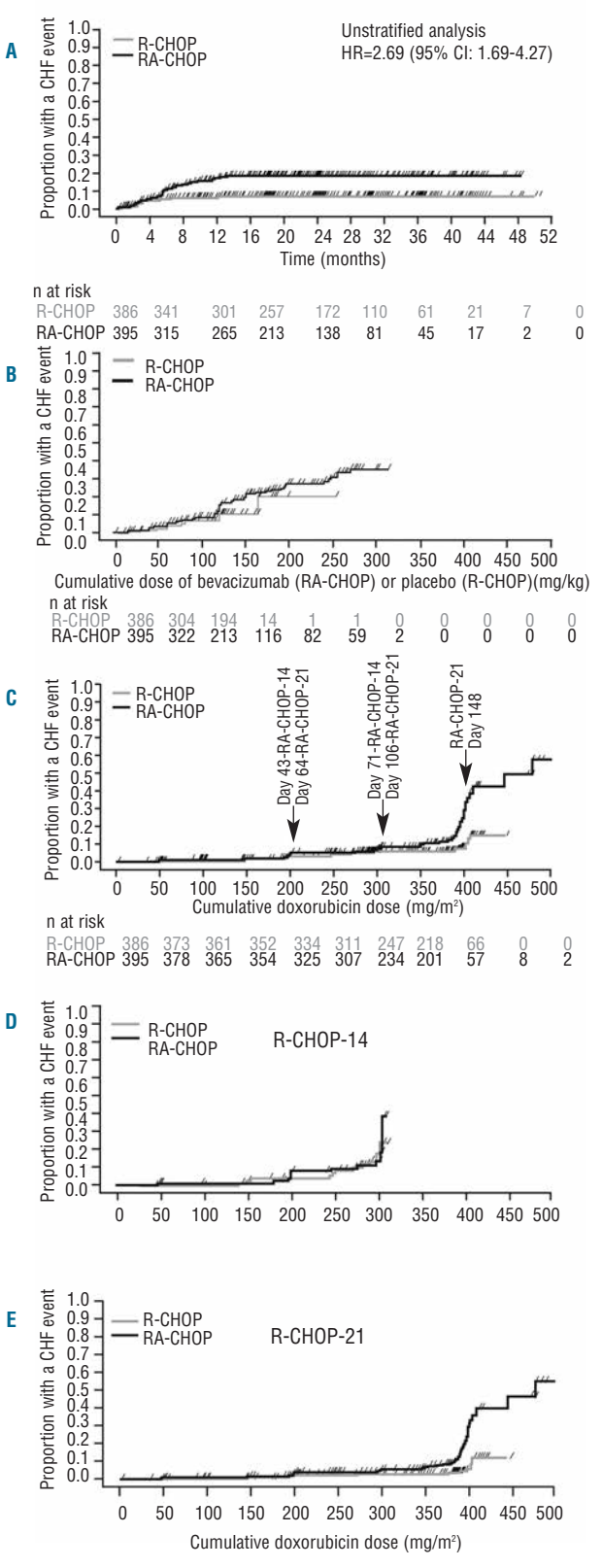

Figure 3. Kaplan-Meier analysis of (A) the time to first CHF event by treatment arm, (B) cumulative bevacizumab dose to first CHF event, (C) cumulative doxorubicin dose to first CHF event, and cumulative doxorubicin dose to first CHF event for (D) R-CHOP-14 and (E) R-CHOP-21 backbone treatments. CHF: congestive heart failure; CI: confidence interval; HR: hazard ratio; R-CHOP: rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone; RA-CHOP: bevacizumab plus rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone.

Table 3. Subgroup analysis of CHF events by treatment arm.

		Patients, n	R-CHOP, %	Patients, n	RA-CHOP, %	Odds ratio	95% CI for odds ratio
All		386	6.5	395	16.2	2.79	1.72–4.54
Chemotherapy backbone	R-CHOP-14	80	13.8	80	13.8	1.00	0.41–2.46
	R-CHOP-21	306	4.6	315	16.8	4.22	2.29–7.78
Age	<65 years	236	4.7	250	14.0	3.33	1.65–6.72
	≥65 years	150	9.3	145	20.0	2.43	1.23–4.81
Sex	Male	201	6.0	185	15.1	2.81	1.38–5.71
	Female	185	7.0	210	17.1	2.74	1.40–5.34
Thoracic radiotherapy	Yes	76	3.9	67	19.4	5.86	1.59–21.58
	No	310	7.1	328	15.5	2.41	1.42–4.08
History of hypertension	Yes	126	9.5	133	19.5	2.31	1.11–4.81
	No	260	5.0	262	14.5	3.22	1.67–6.21
Hypertension as AE	Yes	14	14.3	66	13.6	0.95	0.18–4.95
	No	372	6.2	329	16.7	3.05	1.83–5.08
Base-line LVEF	<60%	68	14.7	71	23.9	1.83	0.77–4.33
	≥60%	317	4.7	321	14.6	3.45	1.89–6.32

CI: confidence interval; AE: adverse event; CHF: congestive heart failure; CI: confidence interval; LVEF: left ventricular ejection fraction; OR: odds ratio; R-CHOP: rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone; RA-CHOP: bevacizumab plus rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone.

function (R-CHOP, 1%; RA-CHOP, 4%). Among the 165 patients who died, the most common cause was disease progression (R-CHOP, n=49; RA-CHOP, n=38). Fatal AEs affected 18 and 32 patients in the R-CHOP and RA-CHOP arms, respectively. Fifteen deaths in the R-CHOP arm and 16 deaths in the RA-CHOP arm were considered by investigators to be related to study treatment. The most common treatment-related AEs leading to death were septic shock (n=6) and pneumonia/bronchopneumonia (n=5).

As expected, AEs of 'special interest' to the known safety profile of bevacizumab were approximately twice as common in patients randomized to RA-CHOP than to R-CHOP, mainly due to the 4-fold higher incidence of hypertension and approximately 2-fold higher incidence of bleeding, CHF, and gastrointestinal perforation (Table 2). The majority of bleeding events in both arms were grade 1/2, with grade 3 or over events reported in 0.3% and 2.0% of patients in the R-CHOP and RA-CHOP arms, respectively. Intracranial bleeding events occurred in 3 patients treated with R-CHOP and in one patient treated with RA-CHOP. Relative to patients in the R-CHOP arm, individuals in the RA-CHOP arm were at higher risk of LVEF AEs (18% *vs.* 8%; OR=2.51; 95%CI: 1.60-3.93). This risk increased over time, particularly after six months of treatment with bevacizumab (*data not shown*). Overall, RA-CHOP-treated patients were more likely to experience CHF events (16% *vs.* 6%; OR=2.79; 95%CI: 1.72-4.54). Although the rate of CHF events was similar for R-CHOP and RA-CHOP over the first 4-5 months of treatment, it plateaued for R-CHOP after month 5, but continued to increase until month 12 for RA-CHOP (Figure 3A). The divergence in CHF event rate between the treatment arms emerged at a cumulative bevacizumab dose of 110-150 mg/kg (Figure 3B). CHF risk was also affected by doxorubicin dose; CHF events began to increase at a cumulative doxorubicin dose of ≥200 mg/m^2 in both treatment arms, with a further increase in risk at a cumulative doxorubicin dose ≥300 mg/m^2 among RA-CHOP-treated patients (Figure 3C). An exploratory analysis revealed differences related to treatment schedule. For patients on a 14-day cycle, the rate of CHF incidence relative to cumulative doxorubicin dose overlapped (Figure 3D), while there was separation of the curves by treatment group at cumulative doxorubicin doses over 300 mg/m^2 for patients on a 21-day cycle (Figure 3E). The frequency of CHF events differed for R CHOP and RA-CHOP across the patient subgroups investigated. In particular, patients aged under 65 years receiving RA-CHOP were 3-fold more likely to experience a CHF event than those receiving R-CHOP (Table 3). Multivariate logistic regression showed that other than treatment (RA-CHOP *vs.* R-CHOP: OR=2.9; 95%CI: 1.8-4.7), the only other covariate that appeared to influence the occurrence of a CHF event was age (≥65 years *vs.* <65 years: OR=1.6; 95%CI: 1.0-2.5).

Table 4. CHF event outcomes by treatment arm.

	R-CHOP (n=386)	RA-CHOP (n=395)
Patients with any CHF (all grade), n (%)*	25 (6)	64 (16)
CHF events, n (%)	27 (7)	68 (17)
Grade ≥3	6 (2)	26 (7)
Outcome, n (%)	(n=27)	(n=68)
Resolved, no sequelae	21 (78)	43 (63)
Resolved, with sequelae	1 (4)	9 (13)
Unresolved	4 (15)	14 (21)
Death	1 (4)	2 (3)
Median duration of CHF, m (95% CI)	3.5 (1.9 - 9.0)	7.8 (5.2 - 12.8)

**Includes CHF events that were reported up to the final cut off. CHF: congestive heart failure; CI: confidence interval; R-CHOP: rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone; RA-CHOP: bevacizumab plus rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone.*

Although CHF events were less likely to resolve without sequelae for RA-CHOP than R-CHOP (63% *vs.* 78%), the rate of CHF events resulting in death was similar (3% *vs.* 4% of patients with a CHF event). However, the median time until resolution of the CHF event was twice as long for patients treated with RA-CHOP *versus* R-CHOP (7.8 *vs.* 3.5 months) (Table 4).

Discussion

The MAIN study demonstrated that the addition of bevacizumab to R-CHOP in patients with DLBCL increases the

risk of LVEF and CHF events relative to standard R-CHOP treatment. The observed increase in cardiac risk occurred without improvement in PFS. In accordance with the recommendation of the DSMB, patient recruitment was stopped and treatment with bevacizumab discontinued. Our findings are supported by the recently published single arm phase II SWOG 0515 trial involving 64 patients with newly diagnosed DLBCL (*clinicaltrials.gov identifier:00121199*). In SWOG 0515, RA-CHOP was also found to lead to an increased incidence of serious toxicities, including cardiac events and gastrointestinal perforations, with actuarial PFS rates comparable to those expected with R-CHOP treatment.[26] Data from the SWOG 0515 trial did not become available until after the discontinuation of bevacizumab treatment in the MAIN study.

Both bevacizumab[27] and doxorubicin[28] can be cardiotoxic. However, we found the addition of bevacizumab to R-CHOP exacerbated the cardiac risk seen with doxorubicin alone, a risk that was also observed in a phase II trial that was published after the current study had been designed and carried out.[26] In both the RA-CHOP and R-CHOP arms, the incidence of CHF events increased beginning at a cumulative doxorubicin dose of 200 mg/m^2. However, while the event rate plateaued in the R-CHOP group despite increasing cumulative doxorubicin doses up to 400 mg/m^2, it continued to increase in the RA-CHOP arm, particularly beyond a cumulative dose of doxorubicin of 300 mg/m^2. This was coincident with a cumulative exposure to bevacizumab of 110-150 mg/kg. A significantly increased risk of CHF with doxorubicin has previously been associated with increased cumulative doses of doxorubicin.[29] Recruitment to an additional single arm phase II trial examining treatment of DLBCL with RA-CHOP (*clinicaltrials.gov identifier:00788606*) was closed after our interim findings became publically available. However, prior to study termination, patients had received median cumulative doses of bevacizumab and doxorubicin equivalent to 120 mg/kg and 300 mg/m^2, respectively. Although there were no instances of symptomatic cardiac failure, 3 of the 6 study participants experienced asymptomatic decreases in LVEF.[30] A similar onset threshold for doxorubicin dose (200 mg/m^2) was observed in a multivariate analysis of the relationship between CHOP and cardiotoxicity in patients with aggressive NHL.[31]

The exact mechanisms underlying doxorubicin- and bevacizumab-induced cardiotoxicity are not known. However, the cardiotoxic effects of doxorubicin have been linked to the generation of reactive oxygen species and oxidative stress, and resulting cardiomyocyte death in pre-clinical studies.[32] Doxorubicin exposure has been shown to stimulate the expression of VEGF in endothelial cells.[33] VEGF, in turn, has a cardioprotective role. It is up-regulated in both animal and human hearts exposed to hypoxic conditions[34-36] and can prevent oxidative stress-induced apoptosis of endothelial cells *in vitro*.[29] Thus, it may be that bevacizumab is not directly toxic to the heart, but rather its inhibition of endothelial VEGF expression prevents the ability of VEGF to counter the negative effects of doxorubicin.

An exploratory analysis of the MAIN study indicated that the rate of CHF events differed according to treatment schedule. CHF incidence was identical (14%) for patients administered chemotherapy every 14 days for six cycles, irrespective of concomitant treatment with bevacizumab, which resulted in a maximum cumulative doxorubicin dose of 300 mg/m^2. Among patients administered chemotherapy every 21 days for eight cycles, which resulted in a maximum cumulative doxorubicin dose of 400 mg/m^2, the rate of observed CHF events was 3.4-fold higher in the RA-CHOP (17%) *versus* R-CHOP (5%) arm, with increased incidence of CHF markedly apparent at cumulative doxorubicin doses of over 300 mg/m^2. However, direct comparison of the 14- and 21-day cycles with respect to CHF is confounded by differences in sample size (n=80 for each R(A)-CHOP-14 group and n>300 for each R(A)-CHOP-21 group), and age of the patients (45% of patients were ≥65 years old in the R(A)-CHOP-14 group compared with 35% in the R(A)-CHOP-21 group), as allocation to these groups was not randomized. Length of cycle was not found to be a significant factor for occurrence of CHF events during multivariate analysis (P=0.4).

The median time to resolution of a CHF event was twice as long for those that developed after treatment with RA-CHOP than those that occurred after treatment with R-CHOP. Although doxorubicin is generally believed to induce irreversible cardiotoxicity, 63% of RA-CHOP-treated patients in our study experienced only short-term transient impairment of LVEF that normalized spontaneously. This phenomenon has also been observed in a single-arm phase II study of patients with DLBCL treated with RA-CHOP.[30] While it is unknown whether LVEF normalization is predictive of truly normalized cardiac function, together these data suggest that at least some instances of bevacizumab-associated cardiotoxicity may be potentially reversible.

Our findings, together with those of others,[29-37] do not support further evaluation of combination treatment with bevacizumab plus R-CHOP in patients with DLBCL. Indeed, it has been suggested that the tumor microenvironment may be predictive of a response to bevacizumab in DLBCL, and that bevacizumab may only be beneficial in DLBCL with high relative expression of a suite of endothelial markers and angiogenic regulators (the 'stromal-2' signature) that is associated with increased tumor blood vessel density.[38]

However, investigations into alternate antiangiogenic therapies in DLBCL may still be warranted provided that their mechanism of action and potential interactions with anthracyclines are thoroughly explored and understood prior to the initiation of large-scale clinical studies. In the interim, translational studies will explore pathological correlates of angiogenesis and will seek to distinguish subgroups of patients with differential outcomes following bevacizumab treatment. Biomarker studies are also underway and are attempting to identify biological correlates that can adequately predict which patients are likely to experience treatment-related cardiotoxicity.

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