R-ESHAP as salvage therapy for patients with relapsed or refractory diffuse large B-cell lymphoma: the influence of prior exposure to rituximab on outcome. A GEL/TAMO study

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ABSTRACT

Background
The role of re-treatment with rituximab in aggressive B-cell lymphomas still needs to be defined. This study evaluated the influence of prior exposure to rituximab on response rates and survival in patients with diffuse large B-cell lymphoma treated with rituximab plus etoposide, cytarabine, cisplatinum and methylprednisolone (R-ESHAP).

Design and Methods
We retrospectively analyzed 163 patients with relapsed or refractory diffuse large B-cell lymphoma who received R-ESHAP as salvage therapy with a curative purpose. Patients were divided into two groups according to whether rituximab had been administered (n=94, "R+" group) or not (n=69, "R-" group) prior to R-ESHAP.

Results
Response rates were significantly higher in the R- group in the univariate but not in the multivariate analysis. In the analysis restricted to the R+ group, we observed very low complete remission and overall response rates in patients with primary refractory disease (8% and 33%, respectively), as compared to those in patients who were in first partial remission (41% and 86%) or who had relapsed disease (50% and 75%) (p<0.01 in both cases). Overall, 60% and 65% of patients in the R+ and R- groups, respectively, underwent stem-cell transplantation after the salvage therapy. With a median follow-up of 29 months (range, 6-84), patients in the R+ group had significantly worse progression-free survival (17% *vs.* 57% at 3 years, p<0.0001) and overall survival (38% v 67% at 3 years, p=0.0005) than patients in the R- group. Prior exposure to rituximab was also an independent adverse prognostic factor for both progression-free survival (RR: 2.0; 95% CI: 1.2-3.3, p=0.008) and overall survival (RR: 2.2; 95% CI: 1.3-3.9, p=0.004).

Conclusions
R-ESHAP was associated with a high response rate in patients who were not refractory to upfront rituximab-based chemotherapy. However, the survival outcome was poor for patients previously exposed to rituximab, as compared to in those who had not previously been treated with rituximab.

Key words: R-ESHAP, salvage therapy, diffuse large B-cell lymphoma, rituximab.

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Introduction

To date, high-dose therapy followed by autologous stem-cell transplantation (ASCT) is the reference treatment for patients with relapsed or primary refractory aggressive non-Hodgkin's lymphoma, provided that the disease is sensitive to second-line chemotherapy.[1-3] Among patients with chemosensitive disease, the remission status at transplant has a significant impact on the outcome, because patients in complete remission before high-dose therapy achieve better long-term progression-free survival than patients who undergo transplantation in partial remission.[4,5] Standard salvage chemotherapy for aggressive lymphoma does not exist. Commonly used second-line regimens include DHAP (dexamethasone, cytarabine, cisplatin), ESHAP (etoposide, methylprednisone, cytarabine, cisplatin), mini-BEAM (carmustine, etoposide, cytarabine, melphalan) and ICE (ifosfamide, carboplatin, etoposide). These regimens produce overall response rates of around 60%, and complete remission rates of 25% to 35%.[5-9] More effective salvage regimens are needed in order to maximize the number of patients in complete remission prior to ASCT.

The chimeric anti-CD20 monoclonal antibody rituximab offers new therapeutic options in the treatment of B-cell non-Hodgkin's lymphoma. The addition of rituximab to CHOP or CHOP-like chemotherapy regimens has been found to significantly improve the complete remission rate and survival in patients with untreated diffuse large B-cell lymphoma (DLBCL).[10,11] Increasing evidence suggests that rituximab added to salvage chemotherapy also improves response rates and outcomes in relapsed DLBCL. In a recent randomized phase 3 study, the efficacy of adding rituximab to the DHAP-VIM-DHAP regimen was tested in 239 rituximab-naïve patients with relapsed or primary refractory aggressive $CD20^+$ B-cell non-Hodgkin's lymphoma. In 225 evaluable patients, the addition of rituximab to second-line chemotherapy resulted in a significant improvement of overall response rate (75% versus 54%, p=0.01) and progression-free survival (52% versus 31% at 2 years, p<0.002).[12] Other small phase 2 trials (with a range of 35-55 patients) investigating rituximab in combination with ICE,[13] DHAP[14] or EPOCH[15] have also shown encouraging results. However, the patients in these studies had not been previously exposed to rituximab, while at present, almost all patients with aggressive B-cell non-Hodgkin's lymphoma receive rituximab combined with first-line chemotherapy. In these patients, the role of rituximab in further salvage treatment remains to be determined.

In the present multicenter retrospective study, we analyzed a large series of patients with DLBCL homogeneously treated with rituximab-ESHAP (R-ESHAP) as salvage therapy, with two goals: (i) to investigate the toxicity and efficacy of this regimen; and (ii) to evaluate the influence of prior exposure to rituximab on response rates and outcomes.

Design and Methods

Patients

Twenty-five GEL/TAMO centers participated in this retrospective study. Investigators from each center were required to report all consecutive patients who met the following inclusion criteria: (i) diagnosis of DLBCL; (ii) age between 18 and 70 years; and (iii) exposure to at least one R-ESHAP cycle with a curative purpose. All centers completed an extensive case report form for every eligible patient. Follow-up questionnaires were sent to obtain missing data. The study was approved by the *Complejo Hospitalario de Zamora* Ethic Committee.

Overall, 163 patients diagnosed with relapsed or refractory DLBCL who received R-ESHAP between May 2000 and July 2007 were included in the study. All patients had received at least one anthracycline-containing regimen prior to R-ESHAP, and 56% of the patients had received rituximab in addition to chemotherapy. Patients were divided into two groups according to whether rituximab was administered (n=94, "R+" group) or not (n=69, "R-" group) prior to R-ESHAP.

Salvage protocol

ESHAP chemotherapy was administered on an inpatient basis as previously described.[7,16] Briefly, etoposide was given at a dose of 40 mg/m^2/day (83% of patients) or 60 mg/m^2/day (17% of patients) as a 1 h intravenous infusion from day 1 to day 4, methylprednisolone was administered at a dose of 250-500 mg/day as a 15 min intravenous infusion from day 1 to day 4 or 5 (total dose of 1 g, 2 g, or 2.5 g in 24%, 52%, and 24% of patients, respectively), cisplatin was given at a dose of 25 mg/m^2/day as a continuous infusion from day 1 to day 4, and 2 g/m^2 of cytarabine was given as a 2 h intravenous infusion on day 5. Rituximab (375 mg/m^2) was infused on day 1 (90% of patients) or day 5 (10% of patients), according to standard prescribing guidelines. The regimen was administered every 3 or 4 weeks (36% and 64% of patients, respectively). Patients received a median of three R-ESHAP cycles (range, 1-6).

Definitions, response criteria and toxicity

Disease status at R-ESHAP was classified as follows: *first partial remission*, including all patients who had shown at least a partial remission after the first-line treatment; *primary refractory disease*, including patients who had not shown a response or who had progressive disease after the first-line treatment; *early relapse*, including patients with a complete remission that lasted less than 1 year, and, finally *late relapse*. Response to R-ESHAP was assessed by conventional diagnostic methods, including computed tomography scanning approximately 28 days after the last cycle. Responses were classified according to the International Working Group criteria (Cheson *et al.*, 1999). Toxicity was evaluated according to the National Cancer Institute's Common Toxicity Criteria, version 3.0.

High-dose therapy and stem-cell transplantation

Overall, 101 out of 163 patients underwent autologous (n=98) or allogeneic (n=3) stem-cell transplantation after the salvage therapy. Twenty-four patients received other therapies between R-ESHAP and transplantation, which included other salvage regimens in 20 patients, local radiotherapy in four patients, and surgical tumor resection in one patient. Reasons for not performing transplantation after the salvage therapy were: the presence of chemoresistant disease (28 patients), poor medical condition of the patient (10 patients), previous ASCT (8 patients), patient refusal (5 patients), death of the patient (4 patients), insufficient $CD34^+$ cell count (3 patients), and the presence of localized disease (3 patients).

End-point definitions and statistical analysis

End-points were assessed on the date of the last contact with the patient; the most recent follow-up was in November 2007. Analyses focused on response rates to R-ESHAP, incidence of grade 2-4 toxicities, progression-free survival and overall survival. Progression-free survival was calculated from the beginning of the first R-ESHAP course until the date of relapse, progression or death from any cause. Overall survival (OS) was calculated from the beginning of salvage therapy to the date of death or the last follow-up. Chi-square test statistics and non-parametric Mann-Whitney tests were used to compare qualitative and quantitative parameters, respectively, between the R+ and R- groups. For binary outcomes such as response rates and toxicity, the differences between the two groups were estimated using the chi square test. Logistic regression analysis was used to adjust the potential effects of other prognostic factors. Survival analyses were performed according to the Kaplan-Meier method. Differences in survival between the R+ and R- groups were analyzed by the log-rank test. A multivariate Cox model was also used to adjust for the potential effects of other prognostic factors with a possible impact on these outcomes. At present, all patients with DLBCL receive rituximab combined with first-line chemotherapy; for this reason, multivariate analysis restricted to patients in the R+ group was also performed. The statistical analyses were computed using SPSS statistical software (SPSS, Inc, Chicago, IL, USA).

Results

Patients' characteristics

The characteristics of the patients included in the study are listed in Table 1. Patients in the R- group received R-ESHAP at an earlier period than those in the R+ group. Patients who had not receveid prior rituximab had been more heavily pretreated: more patients had received two or more prior treatment lines and more patients had undergone an ASCT prior to R-ESHAP. The patient's characteristics were well-balanced between the two groups with regards to sex, age, disease status at R-ESHAP, extranodal disease, bulky disease, β_2 microglobulin level, and age-adjusted International Prognostic Index (aaIPI) (Table 1). B symptoms were more frequently present among patients in the R+ group than among those in the R- group. With regards to the characteristics of the R-ESHAP regimen, no significant differences were observed between the two groups in the doses of etoposide and methylprednisolone administered or in the interval between cycles, as shown in Table 2. However, patients in the R- group received more R-ESHAP cycles: 72.5% of these patients received three or more cycles, as compared to 50% of patients in the R+ group (p=0.004). A similar proportion of patients in both groups underwent transplantation after salvage therapy. There were no significant differences with respect to the conditioning regimens for stem-cell transplantation or the number of $CD34^+$ cells infused (Table 2). None of the patients received rituximab as part of ASCT conditioning, nor as maintenance treatment after transplantion.

Table 1. Patients' characteristics at the time of first R-ESHAP.

Characteristic	R+ group N	R+ group %	R- group N	R- group %	p
Total N. of patients	94	57.7	69	42.3	
Period of treatment					
2000-2003	5	5.3	31	44.9	
2004-2007	89	94.7	38	55.1	<0.001
Male sex	49	52.1	44	63.8	>0.1
Age, years:	55		53		
median (range)	(23-70)		(19-70)		>0.1
Older than 60	39	41.5	21	30.4	>0.1
First-line chemotherapy					
CHOP-21	77	81.9	41	59.4	
Mega-CHOP	5	5.3	9	13	
CHOP-14	6	6.4	1	1.4	
ProMACE	0		8	11.6	
CHOEP	5	5.3	2	2.9	
Others	1	1.1	11	15.9	0.001
Prior exposure to radiotherapy	10	10.6	11	15.9	>0.1
Previous ASCT	3	3.2	13	18.8	0.001
N. of prior treatment lines					
1	86	91.5	49	71	
2-3	8	8.5	20	29	0.001
Disease status at R-ESHAP					
Early relapse	26	27.7	14	20.3	
Late relapse	22	23.4	28	40.6	
First partial remission	22	23.4	12	17.4	
Primary refractory disease	24	25.1	15	21.7	>0.1
Extranodal disease	45	47.9	34	49.3	>0.1
B symptoms	25	30.9	9	15.8	0044
Bulky disease	28	30.4	17	24.6	>0.1
β_2 microglobulin above normal	24	30	19	33	>0.1
LDH above normal	40	44	27	41	>0.1
Ann-Arbor stage III-IV	59	62.7	42	60.8	>0.1
Age-adjusted IPI					
0	20	22	15	22.7	
1	35	38.5	30	45.5	
2	29	31.9	17	25.8	
3	7	7.7	4	6.1	>0.1

R+: prior exposure to rituximab; R-: rituximab-naïve; N: number of patients; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; ProMACE, cyclosphosphamide, doxorubicin, etoposide, prednisone, bleomycin, methotrexate, and leucovorin; CHOEP, cyclophosphamide, doxorubicin, vincristine, etoposide, and prednisone; ASCT, autologous stem-cell transplantation; LDH: lactate dehydrogenase; IPI: International Prognostic Index.

Response to R-ESHAP

The overall response rate to R-ESHAP was 73%, with 35% and 10% of the patients achieving complete remission and complete remission/unconfirmed, respectively, and 28% achieving partial remission. As shown in Figure 1 (1A and 1B), patients who attained complete remission unconfirmed had similar progression-free and overall survival rates as those who reached complete remission. For this reason, in subsequent analyses, these cases were all considered as having a complete remission. As shown in Table 3, patients in the R+ group had lower complete remission (37% *vs.* 56%; p=0.015) and overall response (67% v 81%; p=0.045) rates as compared to patients in the R- group. However, these differences were no longer significant after adjusting for all covariates in the model which had an impact on these outcomes (p=0.16 and 0.27, respectively). In the logistic regression analysis (Table 4), the only factors with an independent adverse influence on both complete remission and overall response rates were: the presence of bulky or primary refractory disease, aaIPI higher than 1 at the time of R-ESHAP, and the administration of fewer than three cycles of R-ESHAP. In the analysis restricted to the R+ group (Table 4), these same four characteristics were also the only independent adverse prognostic factors. We observed very low complete and overall response rates in patients who had primary refractory disease at the time of R-ESHAP (8% and 33%, respectively), as compared to those patients who were in first partial remission (41% and 86%, respectively) or who had relapsed disease (50% and 75%, respectively) (Table 4).

Toxicity of R-ESHAP

Myelosuppression was the most prominent adverse effect. As most patients were discharged after receiving the regimen, indirect conclusions on hematologic toxicities were drawn from the requirement for blood cell transfusions. Thus, 46.6% and 30.4% of patients required red blood cell and platelet transfusions, respectively. Febrile neutropenia occurred in 33.5% of the patients. Three patients, all over 60 years old, died due to infectious complications. As shown in Table 3, there

Table 2. R-ESHAP treatment and stem-cell transplantation.

Characteristic	R+ group N	R+ group %	R- group N	R- group %	p
Dose of etoposide					
160 mg/m²	82	87.2	53	76.8	
240 mg/m²	12	12.8	16	23.2	0.082
Dose of methylprednisolone					
1000 mg	22	23.9	15	21.7	
2000 mg	47	51.1	38	55.1	
2500 mg	23	25	16	23.2	>0.1
Interval between cycles					
21 days	28	31.8	27	40.3	
28 days	60	68.2	40	59.7	>0.1
Number of cycles					
1-2	47	50	19	27.5	
≥3	47	50	50	72.5	0.004
Other therapies before SCT	15	26.3	9	20.9	>0.1
High-dose therapy and SCT	56	59.6	45	65.2	>0.1
Conditioning regimens					
BEAM	35	62.5	24	54.5	
BEAC	11	19.6	15	34.5	
TBI ± chemotherapy	3	5.3	3	6.8	
CVB	4	7.1	1	2.3	
Others	3	5.3	1	2.3	>0.1
Number of CD34⁺ cells infused, median (range) x10⁶/kg	3.23 (1.10-23.70)	3.44 (1.17-36.01)			>0.1

R+: prior exposure to rituximab; R-: rituximab-naïve; N: number of patients; SCT: stem-cell transplantation; BEAM, carmustine, etoposide, Ara-C, melphalan; BEAC, carmustine, etoposide, Ara-C, cyclophosphamide; TBI, total body irradiation; CBV, cyclophosphamide, carmustine, etoposide.

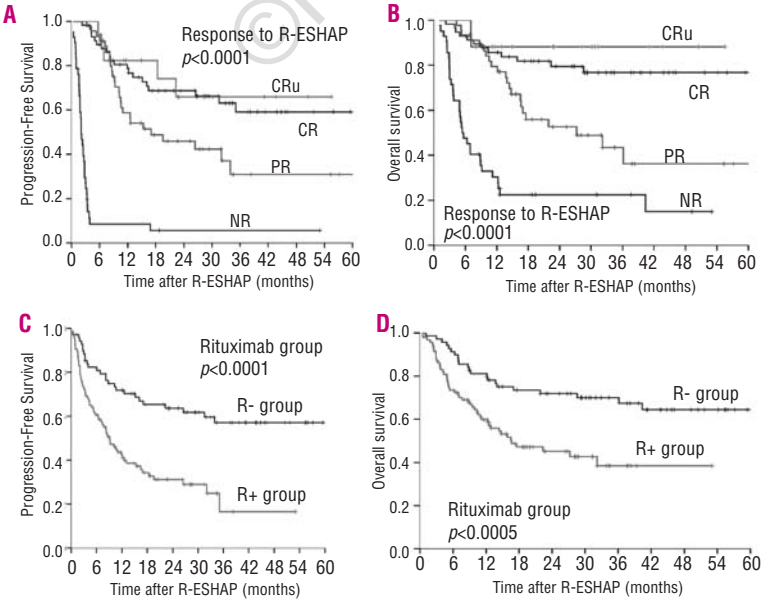

Figure 1. Kaplan-Meier estimation of survival according to response to R-ESHAP: (A) progression-free survival, (B) overall survival, and according to rituximab group: (C) progression-free survival, (D) overall survival. CRu, complete remission unconfirmed; CR, complete remission; PR, partial remission; NR, no response; R+, exposure to rituximab prior to R-ESHAP; R-, rituximab-naïve.

Table 3. Outcomes of the patients in the two rituximab groups.

Outcome	R+ group			R- group			p
	N. of patients evaluated	N	%	N. of patients evaluated	N	%	
Response to R-ESHAP	94			69			0.015
Complete remission		27	28.7		30	43.5	
CR/unconfirmed		8	8.5		9	13	
CR + CRu		35	37.2		39	56.5	0.015
Partial remission		28	29.8		17	24.6	
Overall response rate		63	67		56	81.2	0.045
Treatment-related deaths	94	2	2.1	69	1	1.4	>0.01
Hematological/Infectious toxicity	93			68			
Febrile neutropenia		31	33.3		23	33.8	>0.01
RBC transfusions		43	46.2		32	47.1	>0.01
Platelet transfusions		23	24.7		26	38.2	0.067
Non-hematological toxicity	93			68			
Gastrointestinal (grade 2-4)		5	5.4		11	16.2	0.024
Thrombosis/embolism		4	4.3		0	0	0.084
Metabolic (grade 2-4)		5	5.4		3	4.4	>0.01
Cardiac (grade 2-4)		1	1.1		2	2.9	>0.01
Hepatic (grade 2-4)		1	1.1		1	1.5	>0.01
Neurological (grade 2-4)		1	1.1		1	1.5	>0.01
Renal (grade 2-4)		0	0		1	1.5	>0.01
PFS, probability (95% CI)	94			69			
At 1 year		0.42 (0.31-0.51)			0.72 (0.61-0.83)		
At 3 years		0.17 (0.13-0.32)			0.57 (0.44-0.70)		<0.0001
OS, years, probability (95% CI)	94			69			
At 1 year		0.59 (0.49-0.69)			0.80 (0.70-0.89)		
At 3 years		0.38 (0.25-0.51)			0.67 (0.56-0.79)		0.0005

R+: prior exposure to rituximab; R-: rituximab naïve; N: number of patients; CR: complete remission; CRu: complete remission unconfirmed; PFS: progression-free survival; OS: overall survival.

Table 4. Factors that influence response to R-ESHAP: multivariate analysis.

Factor	Complete remission			Overall response		
	%	RR (95% CI)	p	%	RR (95% CI)	p
Overall series						
Bulky disease at diagnosis						
Yes	31.4			62.9		
No	56.2	2.8 (1.3-6.1)	0.011	80.9	3.5 (1.3-9.5)	0.015
Disease status at R-ESHAP						
Relapsed disease	61.1	6.7 (2.3-19.7)	0.001	83.3	7.4 (2.3-24.1)	0.001
First partial remission	32.4			79.4	6.3 (1.6-24.1)	0.007
Primary refractory disease	20.5			43.6		
Age-adjusted IPI at R-ESHAP						
0	68.6	32.9 (2.7-395)	0.006	88.6	32.0 (3.8-270)	0.001
1	46.2	8.4 (0.8-88.7)	0.077	84.6	19.2 (2.8-129)	0.002
2	34.8			56.5		
3	9.1			27.3		
N. of R-ESHAP cycles						
1-2	27.3			51.5		
≥ 3	57.7	2.7 (1.2-6.1)	0.015	87.6	6.3 (2.3-17.3)	<0.0001
Analysis restricted to R+ group						
Bulky disease at diagnosis						
Yes	19.5			53.7		
No	51	3.9 (1.3-11.7)	0.017	78.4	6.6 (1.5-29)	0.013
Disease status at R-ESHAP						
Relapsed disease	50	11.6 (1.3-100)	0.026	75	3.6 (0.8-16.3)	0.090
First partial remission	40.9	8.8 (0.9-85.4)	0.061	86.4	10.7 (1.5-76.3)	0.01
Primary refractory disease	8.3			33.3		
Age-adjusted IPI at R-ESHAP						
0	60	11.0 (0.7-179)	0.092	85	38.6 (2.5-584)	0.008
1	37.1			82.9	35.7 (2.6-488)	0.007
2	31			48.3		
3	14.3			28.6		
N. of R-ESHAP cycles						
1-2	21.3			44.7		
≥3	53.2	3.1 (1.1-9.1)	0.037	89.4	11.9 (2.4-57.8)	0.002

RR: relative risk; R+: exposure to rituximab prior to R-ESHAP.

were no significant differences in transfusion requirements, incidence of febrile neutropenia or treatment-related deaths between the R+ and R- groups. As regards non-hematologic toxicity, gastrointestinal toxicity was more frequent among patients in the R- group (16.2% v 5.4%; p=0.024), but it was mostly grade 2, consisting of vomiting, mucositis or diarrea. Metabolic toxicity (symptomatic hypocalcemia and/or hypomagnesemia) was reported in approximately 5% of patients in each group. The incidence of other toxicities was under 5% in both groups (Table 3).

Survival analysis

After a median follow-up for surviving patients of 29 months (range, 6 to 83), 91 patients were still alive (44 in the R+ group, 46.8%; 47 in the R- group, 68.1%); 47.2% of the patients experienced relapse or progression (57.4% and 33.3% in the R+ and R- groups, respectively) at a median time of 5.4 months (range, 0.3 to 35.1) after the administration of R-ESHAP. The actuarial 5-year progression-free and overall survival rates were 38% (95% CI, 28% to 46%) and 50% (95% CI, 41% to 59%), respectively. Patients in the R+ group had a significantly worse progression-free survival (17% v 57% at 3 years) and overall survival (38% v 67% at 3 years) as compared to patients in the R- group (Table 3, Figure 1C and 1D).

Prior exposure to rituximab was an independent adverse prognostic factor for both progression-free survival (RR: 2.0; 95% CI: 1.2-3.3; p=0.008) and overall survival (RR: 2.2; 95% CI: 1.3-3.9; p=0.004) (Table 5). According to the multivariate analysis (Table 5), other factors significantly associated with a poor progression-free survival were: the presence of primary refractory disease, an aaIPI higher than 0 and absence of response to R-ESHAP; while an aaIPI higher than 0, the absence of complete remission after R-ESHAP and a failure to perform stem-cell transplantation after the salvage therapy were independent adverse prognostic factors for overall survival. In the analysis restricted to the R+ group (Table 5), aaIPI and the response to R-ESHAP were also the main factors affecting both progression-free survival and overall survival.

Interestingly, the performance of ASCT prior to R-ESHAP did not have a significant influence on survival. Thirteen out of 16 patients treated with ASCT prior to R-ESHAP had not previously been exposed to rituximab. In these patients, progression-free and overall survival rates (both estimated at 5 years) were 74% and 83%, respectively. By contrast, the remaining three patients who had previously been treated with rituximab died within the first year after administration of R-ESHAP.

Table 5. Multivariate analysis of prognostic factors for survival.

	Progression-free survival			*Overall survival*		
Factor	*RR*	*95% CI*	*p*	*RR*	*95% CI*	*p*
Overall series						
Prior exposure to rituximab	2.00	1.20-3.34	0.008	2.23	1.29-3.86	0.004
Disease status at R-ESHAP						
Primary refractory disease	1.98	1.13-3.46	0.017			
Age-adjusted IPI at R-ESHAP						
1	3.40	1.53-7.59	0.003	7.30	1.72-31.03	0.007
2	4.38	1.92-9.99	<0.0001	9.71	2.26-41.59	0.002
3	5.42	1.94-15.15	0.001	23.52	4.87-113.56	<0.0001
Response to R-ESHAP						
Partial remission	1.34	0.73-2.47	0.349	2.65	1.25-5.63	0.011
Non-response	14.27	7.06-28.82	<0.0001	6.04	2.97-12.30	<0.0001
SCT after salvage therapy						
No				2.19	1.24-3.86	0.007
Analysis restricted to R+ group						
Sex male				2.25	1.12-4.50	0.022
Disease status at R-ESHAP						
Primary refractory disease	2.32	1.15-4.68	0.019			
Age-adjusted IPI at R-ESHAP						
1	3.86	1.52-9.77	0.004	11.36	1.50-86.25	0.019
2	3.99	1.53-10.44	0.005	14.62	1.92-111.52	0.010
3	5.28	1.57-17.67	0.007	32.34	3.65-286.54	0.002
Response to R-ESHAP						
Partial remission	0.86	0.39-1.86	0.698	1.89	0.78-4.62	0.166
Non-response	7.13	3.16-16.07	<0.0001	5.32	2.27-12.46	<0.0001
SCT after salvage therapy						
No				2.65	1.30-5.41	0.007

RR: relative risk; R+: exposure to rituximab prior to R-ESHAP; SCT: stem-cell transplantation.

Discussion

Several studies have shown that when rituximab is added to salvage regimens such as DHAP/VIM/DHAP[12] or ICE[13] response rates and progression-free survival in patients with relapsed or refractory DLBCL are improved. However, the patients in these studies had not previously been exposed to rituximab. The role of re-treatment with rituximab in aggressive B-cell lymphomas still needs to be defined. In the present retrospective study, we analyzed a relatively large number of patients with DLBCL who were treated with R-ESHAP. This study is the first comparative analysis of the efficacy and toxicity of a salvage regimen combined with rituximab in patients previously treated with rituximab and patients who were rituximab-naïve. Although this study, like other retrospective multicenter analyses, may have serious shortcomings, the difficulty of designing a prospective study within this setting, due to the current absence of rituximab-naïve patients, increases the interest of the data presented here.

In our study, prior exposure to rituximab did not have an independent effect on response rates to R-ESHAP or toxicity. A relevant finding within the cohort of patients previously exposed to rituximab was that, while patients with primary refractory disease at the time of R-ESHAP had very low complete and overall response

rates (8% and 33%, respectively), those patients who were in their first partial remission or who had relapsed disease showed a high response rate, similar to that of rituximab-naïve patients. However, a high proportion (57.4%) of patients in the R+ group experienced disease relapse or progression, which translated into a significantly worse progression-free survival (17% v 57% at 3 years) and overall survival (38% *vs.* 67% at 3 years) as compared with patients in the R- group. This observation was independent of other prognostic factors with an impact on these outcomes, such as disease status at the time of administering R-ESHAP, aaIPI or response to R-ESHAP. These results are all the more remarkable considering that the R- patients had been more heavily pretreated: more of these patients had received two or more prior treatment lines (29% v 8.5%; p=0.001) and more had undergone ASCT prior to R-ESHAP (18.8% v 3.2%; p=0.001).

There are very few other reports evaluating the efficacy of rituximab re-treatment in aggressive lymphomas. El Gnaoui *et al.*[18] investigated the efficacy of R-GemOx (rituximab, gemcitabine and oxaliplatin) in 46 patients with relapsed or refractory B-cell lymphoma (33 of whom had DLBCL) who were not candidates for high-dose therapy. It is noteworthy that for the 19 responders not previously treated with rituximab, the probability of remaining relapse-free at 2 years was 81%, compared with 37% for the 19 patients previously treated with rituximab. Preliminary results of the CORAL randomized trial comparing R-ICE with R-DHAP in patients with relapsed or refractory DLBCL (only reported in abstract form) also suggest that exposure to rituximab prior to salvage therapy is associated with a worse outcome.[19] Rituximab-resistance has also been described in follicular lymphomas: Davis *et al.*[20] showed that only 40% of patients who had responded to rituximab responded again when retreated with this monoclonal antibody, even though all cases had been ascertained to express CD20 upon enrolment into the study.

The poor survival outcome observed in patients previously exposed to rituximab suggests that the use of highly effective rituximab-containing primary therapy in DLBCL will require salvage therapies based on novel strategies with the capacity to overcome rituximab resistance. Whether this resistance is due to an adaptive property of the malignant B-cell or to impaired immune effector mechanisms in the host remains unclear. Regardless of the causes, rituximab resistance represents a significant barrier in the treatment of B-cell lymphomas. Several approaches to overcome this resistance are under evaluation, including drugs that interfere with intrinsic tumor-related resistance mechanisms and the development of a newer generation of monoclonal antibodies.[21,22]

As far as concerns other relevant prognostic factors, response to R-ESHAP was one of the most important predictive factors for survival, with the best results for patients achieving complete remission (Figure 1A and 1B). The most significant adverse prognostic factors for response in both the whole series and the R+ group were the presence of bulky disease, primary refractory disease, an aaIPI higher than 1 at the time of R-ESHAP, as well as the administration of fewer than three cycles of R-ESHAP. Moreover, the presence of primary refractory disease and high-risk aaIPI at the time of R-ESHAP were also independent adverse prognostic factors for survival, in accordance with reports from other authors,[9,13,14,23-26] but in contrast to the data published by Kewalramani *et al.*,[13] who observed that the addition of rituximab to the ICE regimen seemed to overcome the adverse effects of an unfavorable IPI score. The dismal outcome of patients with primary refractory disease or with an unfavorable aaIPI at the time of relapse underlines the need for the evaluation of alternative treatments. Concerning the number or R-ESHAP cycles, we observed better response rates in patients who received three or more cycles, than in patients who received fewer than three cycles. The explanation for this is that response is usually evaluated after the second cycle, and the following cycles are only administered to patients who are found to have responded to the regimen.

R-ESHAP was well tolerated, myelosuppression being the most prominent adverse effect. The toxicity profile was similar to that previously described for the ESHAP regimen[3] and for other salvage regimens containing platinum-like R-DHAP.[14] Prior exposure to rituximab did not increase toxicity.

In summary, our results show the safety and efficacy of R-ESHAP prior to ASCT in patients with refractory or relapsed DLBCL. A significant number of patients who were not refractory to upfront rituximab-based chemotherapy responded again to rituximab retreatment. However, the progression-free and overall survival rates were significantly worse in patients previously exposed to rituximab than in patients who were rituximab-naïve. The results of this retrospective analysis suggest that the use of highly effective rituximab-containing primary therapy in DLBCL makes it more difficult to salvage patients who are refractory or who relapse. Prospective randomized studies comparing salvage regimens, and incorporating new agents, such as engineered antibodies, novel targeted therapies or radioimmunoconjugates, are needed for these patients.

Authorship and Disclosures

AM: designed the research, analyzed the data and wrote the paper. MDC: contributed to the design of the study and interpretation of data. MAC, EGB, MDC: revised the article critically for important intellectual content. All authors contributed to recruitment of patients, acquisition of data, critical revision of the design of the study and interpretation of data. All authors approved the final version of the paper to be published.

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