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Nonmyeloablative Stem Cell Transplantation Is an Effective Therapy for Refractory or Relapsed Hodgkin Lymphoma: Results of a Spanish Prospective Cooperative Protocol

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ABSTRACT

We report the results of reduced-intensity conditioning allogeneic stem cell transplantation (allo-RIC) in patients with advanced Hodgkin lymphoma (HL). Forty patients with relapsed or refractory HL were homogeneously treated with an RIC protocol (fludarabine 150 mg/m^2 intravenously plus melphalan 140 mg/m^2 intravenously) and cyclosporin A and methotrexate as graft-versus-host disease (GVHD) prophylaxis. Twenty-one patients (53%) had received >2 lines of chemotherapy, 23 patients (58%) had received radiotherapy, and 29 patients (73%) had experienced treatment failure with a previous autologous stem cell transplantation. Twenty patients (50%) were allografted in resistant relapse, and 38 patients received hematopoietic cells from an HLA-identical sibling. Five patients (12%) died from early transplant-related mortality (before day +100 after allo-RIC). One-year transplant-related mortality was 25%. Acute GVHD developed in 18 patients (45%). Chronic GVHD developed in 17 (45%) of the 31 evaluable patients. The response rate 3 months after the allo-RIC was 67% (21 [52%] complete remissions and 6 [15%] partial remissions). Eleven patients received donor lymphocyte infusions (DLIs) for disease relapse. The response rate after DLI was 54% (3 complete remissions and 3 partial remissions). Overall survival (OS) and progression-free survival (PFS) were 48% ± 10% and 32% ± 10% at 2 years, respectively. Refractoriness to chemotherapy was the only adverse prognostic factor for both OS (63% ± 12% versus 35% ± 13%; $P = .05$) and PFS (55% ± 16% versus 10% ± 9%; $P = .006$). For patients with failure of a prior autologous hematopoietic stem cell transplantation, results were especially good for those who experienced late relapses (≥12 months: 2-year OS and PFS were 75% ± 16% and 70% ± 18%, respectively). These data suggest that allo-RIC is feasible in heavily pretreated HL patients and has an acceptable early transplant-related mortality. Results are better in patients allografted in sensitive disease. Both responses observed after the development of GVHD and DLI may suggest a graft-versus-HL effect. Allo-RIC has to be considered an effective therapeutic approach for patients who have had treatment failure with a previous autologous hematopoietic stem cell transplantation.

KEY WORDS

Refractory or relapsed Hodgkin lymphoma • Allogeneic stem cell transplantation • Reduced-intensity conditioning

INTRODUCTION

Approximately 80% of patients with advanced Hodgkin lymphoma (HL) who are treated with modern chemotherapy associated or not with irradiation can now be cured [1,2]. Unfortunately, patients who relapse or prove refractory to first-line therapy do significantly worse [3,4]. High-dose chemotherapy/radiotherapy (RT) followed by transplantation of autologous hematopoietic stem cells (ASCT) is the standard of care for all patients with relapsed or refractory HL [5,6].

The role of allogeneic stem cell transplantation (allo-SCT) in patients with relapsed or refractory HL has been highly controversial. Although several series have suggested that allo-SCT seems to be associated with a clinically significant graft-versus-HL effect and a lower relapse rate with respect to ASCT [7-10], retrospective analyses from the international Bone Marrow Transplant Registry [11] and the European Group for Blood and Marrow Transplantation [12] demonstrate disappointing results with allo-SCT in HL because of an extremely high transplant-related mortality (TRM) that is mainly associated with acute and chronic graft-versus-host disease (GVHD) and concomitant infectious episodes.

Reduced-intensity conditioning allo-SCT (allo-RIC) is currently being evaluated in patients with hematologic malignancies who are considered poor candidates for conventional allo-SCT because of age or associated comorbidities [13-15]. The rationale for allo-RIC relies on previous observations that adoptive transfer of alloreactive donor lymphocytes may eradicate refractory or recurrent disease [16-18]. These RIC protocols could be of benefit for relapsed and refractory HL patients by reducing the high TRM classically associated with conventional allo-SCT, thus improving the long-term outcome in this group of poor-prognosis patients. Several groups have already reported their results with allo-RIC in relapsed or refractory HL [19-23]. Most of these series are characterized by a low number of patients and are heterogeneous in terms of nonmyeloablative protocols and GVHD prophylaxis, and follow-up is usually short. To explore the efficacy of this approach, we analyzed the results of a group of 40 patients with relapsed or refractory HL who were treated with a homogeneous RIC regimen and included in a prospective multicenter Spanish protocol.

PATIENTS AND METHODS

Patient Selection and Characteristics

We have analyzed the data of 40 patients with relapsed or refractory HL who underwent a allo-RIC in 13 transplant centers in Spain between June 1999 and January 2004. Eligibility criteria included the diagnosis of primary refractory HL or relapsed HL (relapse after a first complete remission [CR] with poor-prognostic factors at relapse or multiple relapses, including relapse after an ASCT), age ≤65 years, and availability of an HLA-compatible sibling or an HLA-compatible unrelated donor. Additional eligibility requirements included the absence of active bacterial or fungal infections at the time of transplantation and preserved cardiac (ejection fraction ≥35%) and pulmonary (forced vital capacity ≥30%) functions. Patients were not eligible if there was serologic evidence of infection with the human immunodeficiency virus. Patients gave written informed consent for inclusion in the protocol, which was approved by all local ethical review boards as well as the Spanish drug agency.

Clinical characteristics of the patients included in the study at diagnosis, before the allo-SCT, and at transplantation are shown in Tables 1 and 2, respectively. There were 24 male and 16 female patients with a median (range) age at diagnosis of 31 years (16-53 years) and of 35 years (18-55 years) at the time of allo-RIC (8 patients [20%] were ≥45 years old). Of note, the population of patients included in the study had been very heavily pretreated. The median time between diagnosis and allo-RIC was 37 months (range, 11-300 months). Twenty-one patients (53%) had received more than 2 lines of chemotherapy before allogeneic transplantation, 23 patients (58%) had been treated with complementary RT on previously involved areas during first-line therapy, and 29 pa-

Table 1. *Characteristics of the Patients at Diagnosis and before Transplantation*

Characteristic	Data
Sex	
Male	**24 (60)**
Female	**16 (40)**
Age, y, median (range)	**31 (16-53)**
Histological characteristics	
Nodular sclerosis	**27 (88)**
Mixed cellularity	**3 (7)**
Lymphocyte depletion	**2 (5)**
Ann Arbor stage	
I or II	**16 (40)**
III or IV	**24 (60)**
"B" symptoms	**25 (63)**
First-line therapy	
MOPP-like regimens	**5 (12)**
Doxorubicin-containing regimens	**31 (78)**
Other protocols	**4 (10)**
Complementary radiotherapy	**23 (58)**
Previous ASCT	**29 (73)**
ASCT to allo-RIC interval, mo median (range)	**17 (4-146)**
No. lines of therapy before allo-RIC	
0-2 lines	**19 (47)**
>2 lines	**21 (53)**

ASCT indicates autologous stem cell transplantation; allo-RIC, allogeneic stem cell transplantation after a reduced-intensity conditioning protocol.

Data are n (%) unless otherwise noted.

Table 2. *Characteristics of the Patients and Donors at Allo-RIC*

Characteristic	Data
Diagnosis to allo-RIC interval, mo, median (range)	37 (11-300)
Age, y, median (range)	35 (18-55)
≥45 y	8 (20)
BM involvement	3 (7.5)
ECOG status ≥2	6 (15)
Reason for inclusion in the prospective protocol	
Primary refractory disease	4 (10)
First relapse with 1 or more poor-prognostic factors*	2 (5)
≥2 relapses	6 (12)
Relapse after an ASCT	29 (73)
Disease status at allo-RIC	
Sensitive disease	20 (50)
CR	7 (17)
Sensitive relapse	11 (28)
Untreated relapse	2 (5)
Resistant disease	20 (50)
Donor age, y, median (range)	36 (17-63)
Donor sex	
Male	19 (47)
Female	21 (53)
Donor-recipient sex matching	
D (M) → R (M)	11 (28)
D (M) → R (F)	8 (20)
D (F) → R (M)	13 (32)
D (F) → R (F)	8 (20)
CMV status (D and R)	
Both negative	4 (10)
Other combinations	36 (90)
Type of donor	
HLA-compatible sibling	37 (93)
Mismatched sibling	1 (2)
HLA-compatible URD	2 (5)
Stem cell source for allo-RIC	
BM	37 (93)
PB	3 (7)

Data are n (%) unless otherwise noted.

Allo-RIC indicates allogeneic stem cell transplantation after a reduced-intensity conditioning protocol; CR, complete remission; ASCT, autologous stem cell transplantation; ECOG, Eastern Cooperative Oncology Group; URD, unrelated donor; D, donor; R, recipient; BM, bone marrow; PB, peripheral blood.

*Adverse prognostic factors at first relapse: first CR <12 months, stage IV at relapse, B symptoms, and hemoglobin <105 g/L.

tients (73%) had previously experienced a failed ASCT. Of the last group, 10 patients (29%) had a CR after ASCT of >1 year, and the remaining 19 patients (71%) relapsed in the first year after autologous transplantation. There were no previous significant comorbidities in the group of patients included in the protocol.

Conditioning Regimen and GVHD Prophylaxis: Supportive Care—Donor Lymphocyte Infusions after the Allogeneic Procedure

All patients received a homogeneous conditioning regimen that consisted of the combination of fludarabine 30 mg/m^2 intravenously for 5 days followed by melphalan 140 mg/m^2 intravenously divided into 2 doses. In addition, 3 patients, 1 allografted from a 1 antigen–mismatched related donor and 2 allografted from HLA-compatible unrelated donors, received rabbit antilymphocyte globulin (2.5 mg/kg intravenously for 3 consecutive days) as part of the conditioning regimen.

Hematopoietic stem cells were infused on day 0. When peripheral blood (PB) was used as the source of hematopoietic stem cells, leukapheresis were performed on days 0 and 1 if necessary to infuse a minimum number of 2.0×10^6 CD34$^+$ cells per kilogram of body weight to the patient. The median number (range) of PB CD34$^+$ cells infused was 6.5×10^6/kg ($2.1\text{-}14.5 \times 10^6$/kg).

Acute and chronic GVHD were graded by following established criteria [24,25]. GVHD prophylaxis consisted of the combination of cyclosporin A (CsA) 3 mg/kg intravenously starting on day −1. In the absence of grade II or greater acute GVHD, CsA was tapered 10% weekly starting on day +90 after the allo-RIC and was discontinued by day +150. Methotrexate (MTX) was given at 10 mg/m^2 intravenously on days +1, +3, and +6, followed by folinic acid rescue.

All patients were treated in individual rooms with reverse-isolation protective measures during neutropenia. Antimicrobial prophylaxis was administered according to the local standard practice guidelines of the respective institutions.

Patients with relapsed or progressive disease after transplantation or those who did not evolve to 100% donor chimerism in the absence of grade II or higher acute GVHD were treated with rapid discontinuation of systemic immunosuppression to initiate graft-versus-HL effects. In the absence of disease response and grade II or higher acute GVHD, patients were considered eligible for donor lymphocyte infusions (DLIs). An escalated dose regimen starting at 1×10^7 CD3$^+$ T cells per kilogram was used in all patients. Donor lymphocytes were infused at intervals of 2 months up to a dose of 5×10^8 CD3$^+$ T cells per kilogram.

Chimerism Analyses

For chimerism analyses, nucleated cells were isolated from the marrow, T cells, and granulocytes from the PB on days 28, 100, and 180 after the allo-RIC procedure or when necessary (if clinically indicated). Percentages of donor-host chimerism were evaluated by means of a polymerase chain reaction–based amplification of variable number tandem repeat sequences unique to donors and hosts [26,27]. Complete donor chimerism was defined as 100% donor cells, whereas mixed chimerism indicated the presence of ≥1% recipient cells in the sample analyzed.

Study Definitions: Evaluation of Response

Patients were staged according to the Ann Arbor system [28] and radiologically evaluated by means of computed tomographic scan and gallium 67 gammagraphy. Patients were defined as having primary refractory disease if they had received induction chemotherapy, with or without salvage therapy, and did not achieve a partial response (PR) or CR. A sensitive relapse was defined as a reduction of ≥50% of the bidimensional measurements of the disease with the use of conventional salvage chemotherapy or RT. Resistant relapse was defined as a <50% reduction in the size of the tumor with the use of conventional salvage chemotherapy.

Patients were clinically staged at the time of allo-RIC, on day +90 after the procedure, every 6 months for the first 2 years, and then yearly or as clinically indicated. Patients who received DLIs for disease relapse or progression were also evaluated 1 month after DLI. Patients who survived >90 days after allo-RIC without evidence of tumor by clinical and radiologic evaluation were classified as having CR. Patients with small residual radiographic abnormalities that did not progress for 6 months after transplantation were also classified as being in CR. Partial remission was defined as a ≥50% reduction of pretransplantation measurable disease for at least 1 month. Patients who achieved a <50% tumor reduction after ASCT were considered as nonresponders according to the established criteria [29]. Nonhematologic toxicities that developed after the allo-RIC procedure were evaluated according to the World Health Organization common toxicity criteria.

Statistical Methods

Survival analyses were performed according to the Kaplan-Meier method [30]. Overall survival (OS) was calculated in months from the date of autologous stem cell reinfusion to the date of death from any cause, and surviving patients were censored at last follow-up. Disease-free survival was calculated from transplantation until disease progression for patients who achieved a CR after transplantation and also for patients who reached CR after DLI. Patients who experienced TRM and those who were still alive without progression at the time of reporting were censored at death and at last follow-up, respectively. Event-free survival (EFS) was calculated from transplantation until disease progression or death, and patients who did not reach a disease response (CR or PR) at any time after transplantation were considered events. Overall TRM was defined as death from any cause other than HL and was calculated by using a cumulative incidence method. For the purpose of this analysis, TRM was divided into early TRM (death from any cause other than lymphoma within the first 100 days after the allo-RIC) and late TRM (death from any cause other than lymphoma beyond the first 100 days after the allogeneic procedure). Acute GVHD incidence was also calculated by using a cumulative incidence method in patients who survived beyond day +21 after allo-RIC. The incidence and time of onset of chronic GVHD were calculated in patients followed up for at least 90 days.

Comparison of the survival curves in univariate analysis was performed by using the log-rank test [31]. Analysis of prognostic factors influencing both CR and TRM rates was performed by Fisher exact test and logistic regression analysis.

Multivariate analysis was performed by using a forward stepwise Cox proportional hazards model. The prognostic factors analyzed for all survival end points were the time interval between diagnosis and allo-RIC (<36 versus ≥36 months), the number of lines of therapy before transplantation (≤2 versus >2 lines), previous ASCT (no versus yes), complementary RT before allo-RIC (no versus yes), Eastern Cooperative Oncology Group status at allo-RIC (<2 versus ≥2), disease status at allo-RIC (sensitive versus resistant disease), sex mismatch between donor and recipient (female to male versus other combinations), and cytomegalovirus (CMV) serology for both donor and recipient (donor and recipient CMV^- versus other combinations).

All P values reported are 2 sided, and statistical significance was defined as a P value <.05. The statistical analyses were computed with SPSS statistical software (SPSS Inc., Chicago, IL).

RESULTS

Engraftment and Nonhematologic Toxicities

All patients but 1, who did not have recovery of neutrophil counts $>0.5 \times 10^9$/L and was classified as a primary graft failure, presented an early and sustained engraftment after transplantation. The median time for $>0.5 \times 10^9$/L granulocytes in the PB was 15 days (range, 10-26 days), and for 20×10^9/L and 50×10^9/L platelets, the median times were 11 days (range, 8-38 days) and 15 days (range, 11-49 days), respectively.

All the patients except for the 1 who developed a primary graft failure and had an autologous recovery afterward presented with 100% donor chimerism on day +30 after transplantation. Full donor chimerism was observed during the follow-up of all the patients, and no case of secondary or late graft failure was reported in our series.

The most frequently observed nonhematologic toxicity was gastrointestinal; 18 (45%) patients experienced grades II to IV oral mucositis, and 7 (17%) patients required total parenteral nutrition during the

immediate posttransplantation period. Grade II to IV upper gastrointestinal toxicity (nausea and vomiting), mainly related to the conditioning protocol, was observed in 8 (20%) patients. No cardiac, renal, or pulmonary toxicity was observed.

Infectious Complications and GVHD

Thirty-one patients (77%) presented with fever during the neutropenic period; it was documented microbiologically in 14 cases (45%). Early infections included 8 cases of bacteremia, 4 cases of pneumonia (3 of unknown origin), 1 case of endocarditis, 1 case of syncytial respiratory virus infection, and 1 *Escherichia coli* urinary tract infection. Early infections were the immediate cause of death in 4 patients (10%).

Eighteen patients (45%) developed acute GVHD at a median of 21 days (range, 7-69 days) after transplantation. It was greater than grade II in only 3 cases (16%). The cumulative incidence of grades II to IV acute GVHD was 42% (95% confidence interval [CI], 29%-61%). High-dose steroids were used as first-line therapy for acute GVHD in 16 of these 18 patients. The response rate to steroids was 82% and included 11 CRs (65%) and 3 PRs (17%).

Chronic GVHD was seen in 17 (45%) of the 31 evaluable patients. It was limited in 10 cases (59%) and extensive in 7 cases (41%). The cumulative incidence of chronic extensive GVHD at 1 year was 47% (95% CI, 31%-69%).

Transplant-Related Mortality

Five patients (12.5%) died before day 100 after transplantation because of transplant-related causes: 4 patients as a result of severe infections (80%) and 1 patient (20%) as a result of acute GVHD not responsive to therapy. The cumulative incidence of 100-day TRM was 12.5% (95% CI, 5%-28%; Figure 1). Five more patients (14%) died after day 100, basically because of progressive chronic GVHD. The cumulative incidence of 1-year TRM was 25% (95% CI, 13%-43%). Univariate analysis identified complementary RT before the allo-RIC (0% versus 42%; $P = .02$) and an Eastern Cooperative Oncology Group status ≥2 at allo-RIC (32% versus 67%; $P = .05$) as significant prognostic factors (Table 3). Patients with neither risk factor had a 1-year TRM of 0%, whereas in patients with both risk factors (n = 5), TRM was as high as 70% ± 24% at 1 year after allo-RIC. Multivariate analysis identified the use of complementary RT before the allogeneic procedure as the only factor that significantly increased 1-year TRM (Table 4).

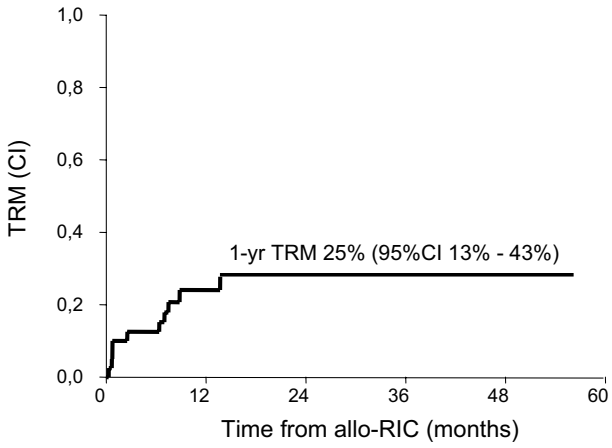

Figure 1. Cumulative incidence of 100-day and 1-year transplant-related mortality.

Table 3. *Univariate Analyses of Prognostic Factors for Overall Survival (OS), Event-Free Survival (EFS), and Progression-Free Survival (PFS) at 2 Years and 1-Year Transplant-Related Mortality (TRM)*

Prognostic Factor	No. Patients	Results at 2 y (95% CI)	*P* Value
OS			
Disease status at allo-RIC			
Sensitive disease	20	63%	.05
Resistant disease	20	35%	
EFS			
Remission duration after ASCT			
<12 mo		33%	.05
≥12 mo		6%	
Disease status at allo-RIC			
Sensitive disease	20	50%	.01
Resistant disease	20	0%	
PFS			
Remission duration after ASCT			
<12 mo		70%	.05
≥12 mo		0%	
Disease status at allo-RIC			
Sensitive disease	20	55%	.007
Resistant disease	20	10%	
TRM			
Complementary RT before allo-RIC			
No		47%	.02
Yes		0%	
ECOG status at allo-RIC			
<2		67%	.05
≥2		32%	

CI indicates confidence interval; allo-RIC, allogeneic stem cell transplantation after a reduced-intensity conditioning protocol; ASCT, autologous stem cell transplantation; RT, radiotherapy; ECOG, Eastern Cooperative Oncology Group.

Donor Lymphocyte Infusions

Eleven patients (27.5%) received at least 1 DLI because of persistent disease or disease progression. The median number of DLIs infused was 2 (range, 1-6), and in 3 cases (27%) the DLI was preceded by the administration of chemotherapy (NOVP [mitoxantrone, vincristine, vinblastine, prednisone] protocol

Table 4. *Adverse Prognostic Factors Influencing Overall Survival (OS), Event-Free Survival (EFS), Progression-Free Survival (PFS), and 1-Year Transplant-Related Mortality (TRM)*

Prognostic Factor	RR	95% CI	*P* Value
OS			
Resistant disease at allo-RIC	**2.3**	**1.0-6.1**	**.05**
EFS			
Resistant disease at allo-RIC	**2.5**	**1.2-5.6**	**.01**
PFS			
Resistant disease at allo-RIC	**3.8**	**1.3-10.9**	**.01**
TRM			
Complementary RT before allo-RIC	**14.8**	**1.5-141.1**	**.01**

RR indicates relative risk; CI, confidence interval; allo-RIC, allogeneic stem cell transplantation after a reduced-intensity conditioning protocol; RT, radiotherapy.

in all 3 patients) to optimize disease control. The response rate after DLI was 54%: 3 patients (27%) achieved a CR, and 3 (27%) achieved a PR. Acute GVHD developed in 5 patients (46%) after DLIs: it was greater than grade II in 2 patients. Chronic GVHD was seen in 5 cases (46%), 3 of whom had previously had acute GVHD.

Disease Responses and Follow-Up

Disease status was evaluated at 3 months after transplantation. At this time point, 22 patients (55%) were in CR or very good PR, 5 patients (13%) were in PR, 4 patients (10%) were in stable disease, 4 (10%) were in progressive disease, and 5 (12%) had died because of early TRM. The median follow-up of the entire series was 260 days (range, 9-1516 days). At the last follow-up, 22 patients (55%) were alive with a median follow-up of 376 days (range, 123-1516 days). Of these, 13 patients (32%) were alive without evidence of disease progression, and 9 patients (23%) had active disease. Eighteen patients (45%) had died, 6 (20%) from disease progression and 10 (25%) from TRM.

When we analyzed the possible effect of the development of acute and chronic GVHD on disease control after transplantation, we could demonstrate a borderline significant difference in terms of progression-free survival (PFS) between patients who developed extensive chronic GVHD and those who did not develop this complication after transplantation (71% ± 17% versus 44% ± 20%; $P = .07$; Figure 2).

Actuarial OS, EFS, and PFS at 2 years were 48% ± 10%, 20% ± 7%, and 32% ± 10%, respectively (Figure 3). The univariate analysis for the 3 survival end points analyzed is shown in Table 4. On multivariate analysis, the presence of chemorefractory disease was the only adverse prognostic factor for OS, EFS, and PFS (Table 4): the 20 patients who allografted with sensitive disease at the time of transplantation presented an actuarial OS, EFS, and PFS at 2 years of 63% ± 12%, 33% ± 13%, and 55% ± 16%, respectively (Figure 4).

More than 75% of the patients in our series were allografted because of relapse after an ASCT. The 1-year TRM was 24%. The 2-year OS, PFS, and EFS were 52% ± 11%, 34% ± 13%, and 23% ± 9%, respectively. The only significant prognostic factor in the multivariate analysis was the duration of the remission after the autologous procedure (>12 versus ≥12 months). Especially good results were obtained in patients allografted for late relapses after an ASCT (n = 10 in our series), with 2-year OS, EFS, and PFS of 75% ± 16%, 50% ± 17%, and 70% ± 18%, respectively (Figure 5).

DISCUSSION

Conventional allo-SCT has proven to be a potentially curative approach for diverse hematologic malignancies. Nevertheless, its availability is limited for reasons of age, availability of an HLA-compatible donor, and the presence of comorbidities in the recipient. Although conventional allo-SCT has been classically associated with an impressively high TRM [11,12], several other reports also indicate a possible existence of a graft-versus-HL effect [7-10], which could be of benefit in terms of long-term disease control in some subgroups of patients who have a very poor outcome with other conventional therapies, such as autologous transplantation.

It has been well established that RIC protocols may offer a reduced risk of procedure-related mortality [13-15,32,33] compared with conventional transplantation, and this may increase the percentage of candidates for the allogeneic procedure by allowing this procedure in older patients and in those with

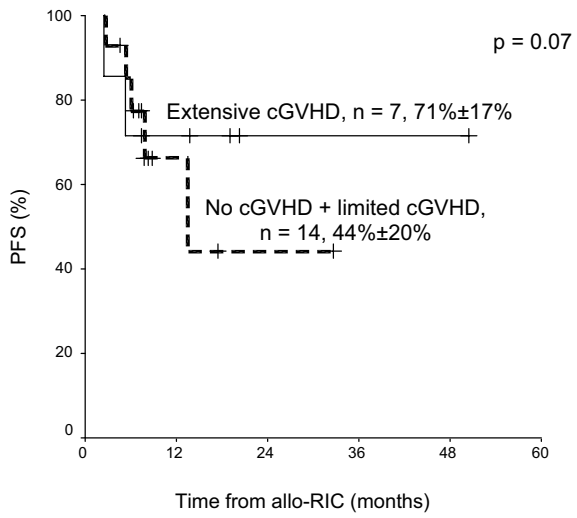

Figure 2. Relationship between progression-free survival (PFS) and patients developing extensive chronic graft-versus-host disease (cGVHD) after transplantation.

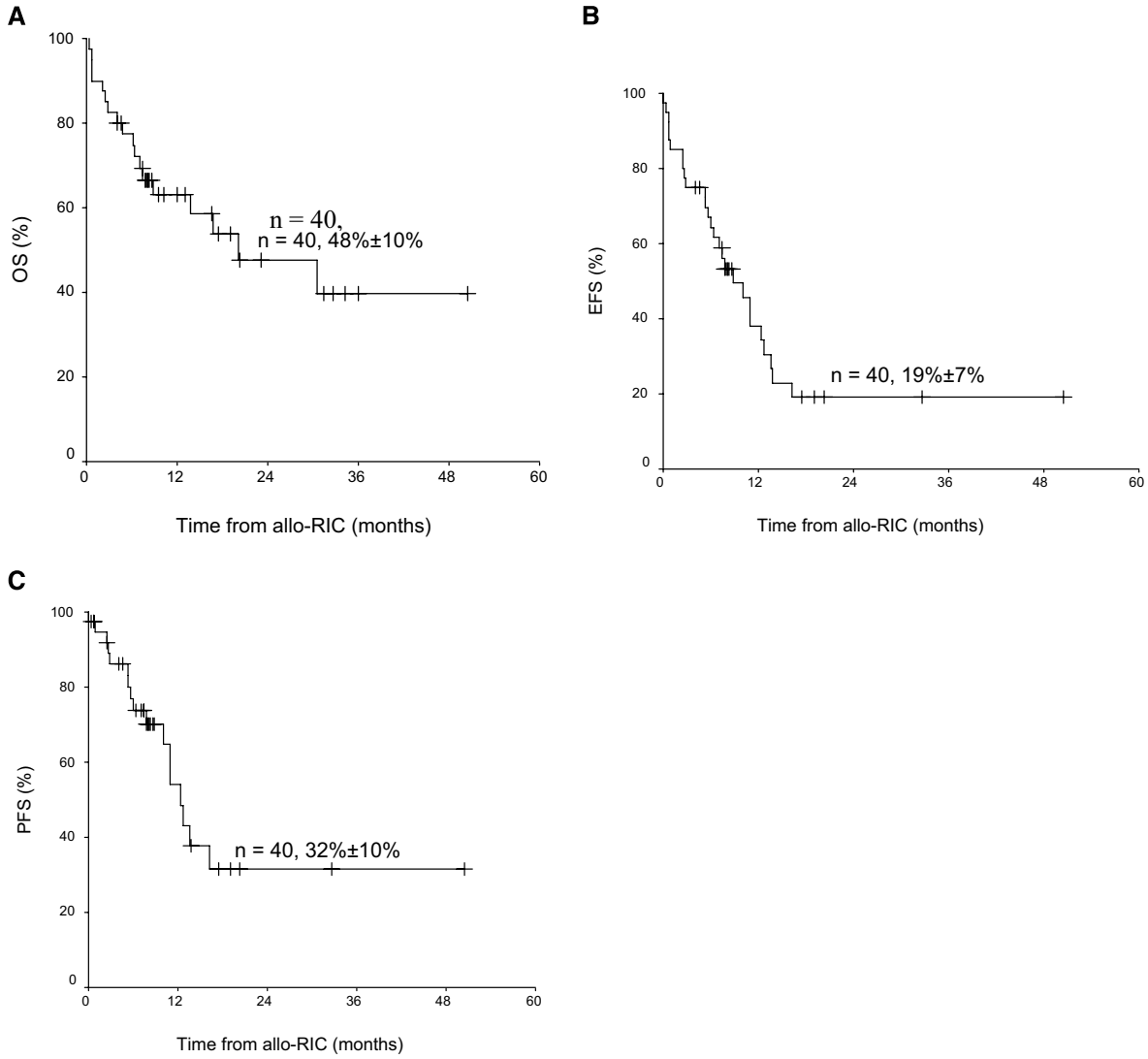

Figure 3. Actuarial 2-year overall survival (OS) (A), event-free survival (EFS) (B), and progression-free survival (PFS) (C) of the entire series.

other significant comorbidities at the time of transplantation [34]. The aim of this approach is to achieve a graft-versus-disease effect based on the alloreactivity of donor T lymphocytes with a reduced TRM while also maintaining some antitumoral activity. In the case of relapsed or refractory HL, RIC procedures could be of benefit in patients with a high risk of relapse by reducing the high TRM associated with the conventional allogeneic procedure and, thus, allowing the emergence of a clinically significant graft-versus-HL effect. In this sense, the number of RIC transplantations has been progressively increasing in Europe in the last 5 years.

With all these thoughts in mind, in 1999 the Spanish Cooperative Group started a prospective protocol to analyze the feasibility of a reduced-intensity protocol in patients with hematologic malignancies who were not candidates for a conventional allo-SCT [33]. Taking into account the extremely poor results shown by the registry analyses [11,12], all patients with HL who fulfilled other inclusion criteria were included in the protocol, independently of age. Although the intensity of the myelosuppression of the RIC regimens widely differs from one protocol to the other [13-15,32], the combination of fludarabine and high-dose melphalan was chosen because its immunosuppressive capacity is enough to allow a full allogeneic hematopoietic engraftment, but, at the same time, it provides a significant antitumoral effect, which may be needed in some clinically aggressive diseases, such as HL, in which the graft-versus-lymphoma effect does not seem potent enough. The chosen GVHD prophylaxis was the classic combination of CsA and short-course MTX. It was thought that the more intensive GVHD prophylaxis combination could abrogate more aggressively the development of acute GVHD after transplantation and, thus, the clinically associated graft-versus-malignancy effect, which is considered to be the basis of this new approach. Allogeneic hematopoietic engraftment has not been a significant pitfall of this

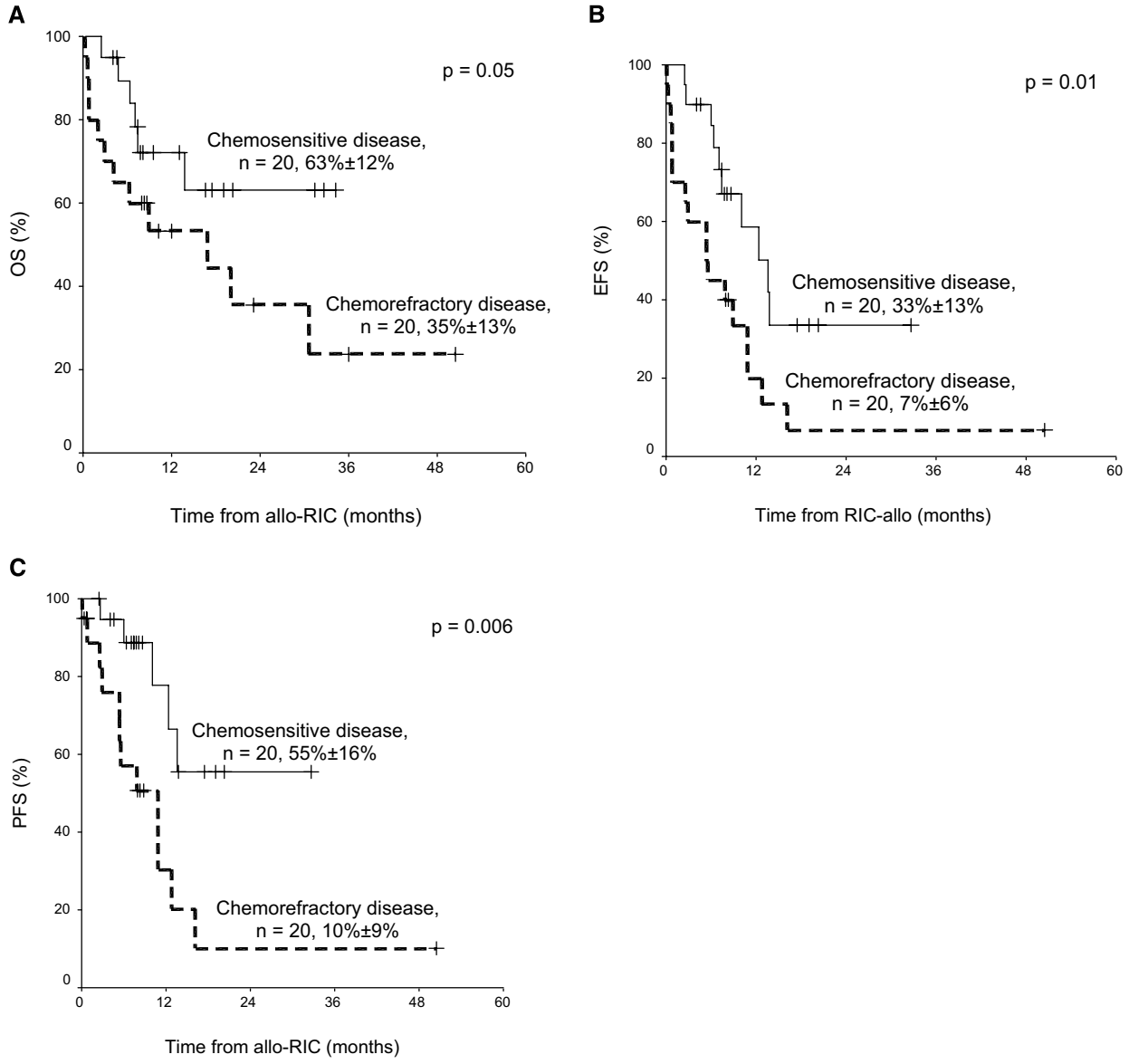

Figure 4. Differences in overall survival (OS) (A), event-free survival (EFS) (B), and progression-free survival (PFS) (C) between chemosensitive (n = 20) and chemorefractory (n = 20) patients.

combination. All the patients included in this trial but 1 showed a full donor chimerism when analyzed on day +28 after transplantation and were maintained as full chimeras during the complete follow-up. This fludarabine-melphalan combination has been previously reported by our group to achieve a high percentage of complete donor chimerism very rapidly after transplantation in relation to the fludarabine-busulfan combination [27], probably in part because of the intense immunosuppression presented by the patients undergoing this conditioning protocol. The M.D. Anderson Cancer Center has also reported a 100% full donor chimerism for HL patients receiving the fludarabine-melphalan association but only a 50% full chimerism for those receiving the association of fludarabine and cyclophosphamide [19]. Only 1 of our patients developed a primary graft failure with an autologous reconstitution demonstrated by means of the polymerase chain reaction technique. This patient had a history of idiopathic thrombocytopenic purpura and Sjögren syndrome; although she was highly immunosuppressed before the allo-RIC, this autoimmune situation could have contributed to the primary graft rejection.

One of the main objectives of RIC protocols is the reduction of transplant-related toxicities and, thus, the possibility of offering an allogeneic procedure to older patients. In our group of patients, 8 (20%) were ≥45 years at the time of transplantation, and, as previously shown by other groups [19-23], in this protocol both 100-day and 1-year TRM (12.5% and 25%, respectively) have been significantly lower than what has

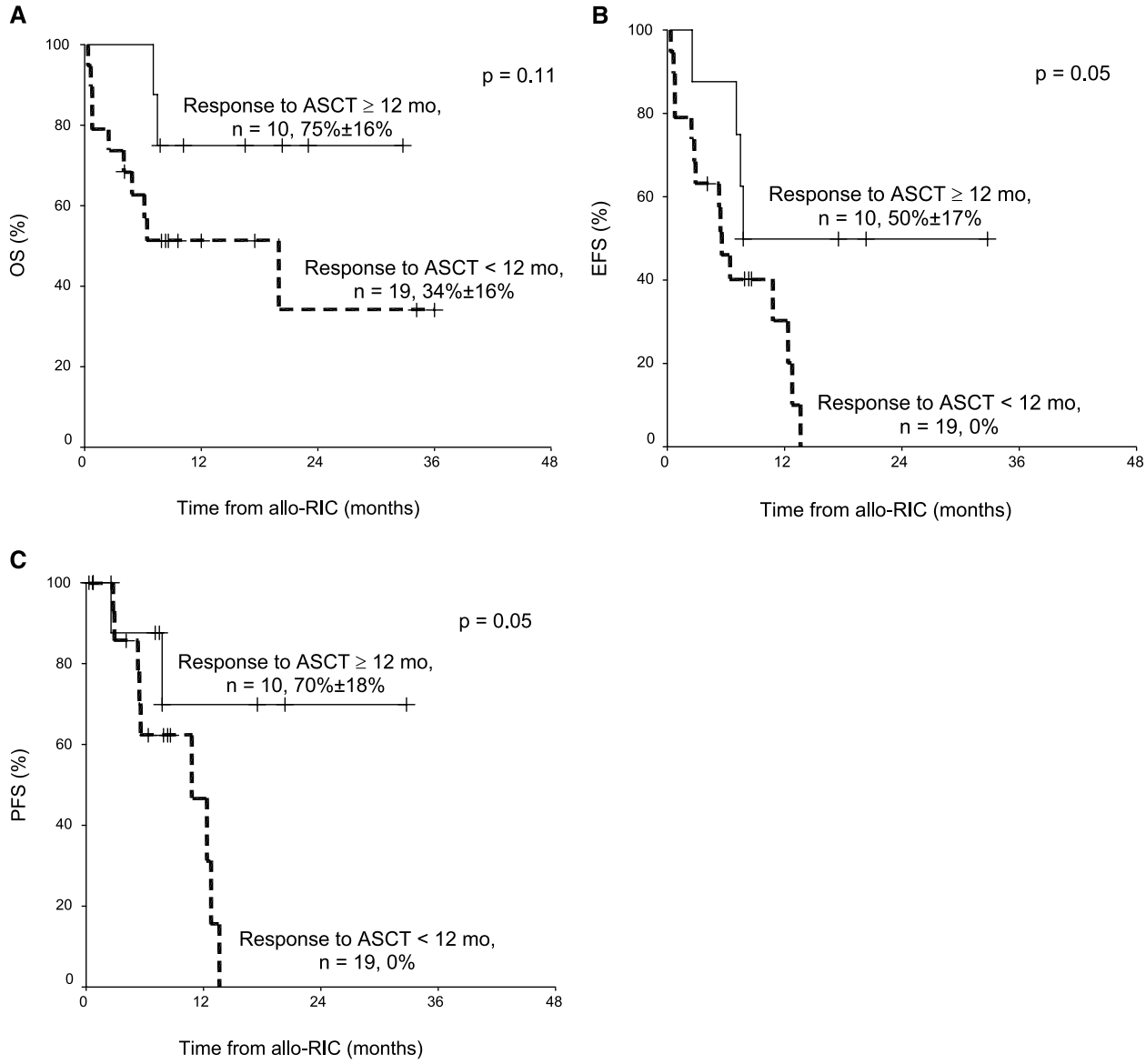

Figure 5. Allo-RIC in patients who had a previously failed ASCT. Differences in overall survival (OS) (A), event-free survival (EFS) (B), and progression-free survival (PFS) (C) are shown between late (n = 10) and early (n = 19) relapses.

been previously reported for conventional allo-SCT in refractory and relapsed HL [11,12]. However, these results will require longer follow-up for proper evaluation. This lower TRM in high-risk HL patients has also been reported by other groups: the M.D. Anderson group reported a 100-day TRM of 8% in a group of 25 patients with high-risk HL who were treated with an RIC protocol consisting of the combination of fludarabine-melphalan or fludarabine-cyclophosphamide, and the Fred Hutchinson Cancer Research Center also reported an early TRM of 8% in a group of 12 patients with HL treated with a less myelosuppressive regimen consisting of the combination of fludarabine and low-dose total body irradiation. This TRM increased to 22% at 1 year, basically because of complications related to the development of GVHD and serious infectious episodes [22]. In our series, fatal respiratory infections, which were more frequently present in patients who had previously received RT, were the leading cause of the significantly higher TRM in this subgroup of patients. Early and late TRM were even more reduced in the context of an RIC protocol developed by the British Cooperative Group that used alemtuzumab as GVHD prophylaxis in combination with CsA. Peggs et al. [23] recently presented the results in a group of 49 patients with high-risk HL treated with an RIC protocol that consisted of the combination of fludarabine and melphalan at the same doses as those in the Spanish Cooperative protocol and the association of CsA at the usual doses plus alemtuzumab. A 4.1% 100-day TRM was observed that increased to 16.3% at 730 days after the allogeneic procedure,

taking into account the deaths directly related to DLIs and their complications.

Although one of the theoretical advantages that was initially associated with RIC protocols was the reduction of the incidence of acute GVHD after transplantation as a result of the reduction of tissue damage produced by the conditioning protocol, this has not been the case in practice. In fact, acute and chronic GVHD and their directly related complications continue to be the leading causes of morbidity and mortality after these protocols [21,22], not only for HL patients, but also for patients with other hematologic malignancies [14,15,33,34]. This higher-than-expected GVHD incidence observed in these RIC protocols might reflect, at least in part, the older age, as well as the amount of therapy previously received before the allogeneic protocol. In our study, acute GVHD was present in 45% of the series, although it was grade III or IV in only 16% of the patients. Chronic GVHD was also seen in a high percentage of the evaluable patients (45%), although it was extensive in only 7 patients (41%). These incidences are similar to those reported by other authors [19,21,22], and these complications also constitute the main cause of mortality in other series [22]. The use of alemtuzumab has also significantly reduced the incidences of both acute and chronic GVHD; the British Cooperative Group has reported a rate of acute GVHD grades II to IV of 16% (8 patients) in their group of 49 patients, and it reported 7 cases of chronic GVHD (14%) (4 limited and 3 extensive) after allo-RIC [23].

Both the somewhat lower incidence of relapse in patients developing extensive chronic GVHD and the disease responses observed after DLIs (54% in our study) suggest the existence of a graft-versus-HL effect, which is the most clinically important weapon of the allogeneic procedure. This graft-versus-HL effect has also been demonstrated in the alemtuzumab setting of the British Cooperative Group trial [23]. In their recent report of 49 patients, they show a PFS of 36.3% for recipients of related allogeneic grafts and 22.6% for recipients of unrelated grafts at 4 years and a 56% disease response to DLIs after transplantation (8 CRs and 1 PR). Clinical responses after DLIs were associated with the development of GVHD after the infusion of lymphocytes.

Although the median OS of the surviving patients of 1 year is still rather short, the 48% ± 10% 2-year OS and the 32% ± 10% 2-year PFS of our series of 40 patients are comparable to what has been published in the literature [19,21-23]. These results were even better when we analyzed separately the patients who were allografted in chemosensitive disease (63% ± 12% and 55% ± 16%, respectively). The disease response to previous salvage chemotherapy has been the only significant prognostic factor for the 3 survival end points analyzed in this study. This has also been indicated by other authors [19,22] in the context of the allo-RIC, and it is one of the major determinant prognostic factors for other salvage therapies, eg, salvage conventional chemotherapy and ASCT used in patients with relapsed or refractory HL. Nevertheless, and in our experience, even in chemorefractory patients, a significant percentage of them (35%) become long-term survivors.

Especially good results, in terms of PFS, OS, and 1-year TRM, have been obtained in patients treated with allo-RIC as salvage therapy for late relapses after ASCT. Similar results were suggested by Branson et al. [35] in a group of 38 patients with lymphoproliferative disorders treated with a Campath 1H (Schering AG, Berlin, Germany) RIC protocol after a previous ASCT failure.

The real effect of the allo-RIC procedure in relapsed or refractory HL is still unknown. It seems that it clearly reduces TRM with respect to conventional allo-SCT in this disease and, thus, improves outcome in a similar subset of patients with a very bad prognosis. A retrospective analysis of the Lymphoma Working Party of the European Group for Blood and Marrow Transplantation comparing results between these procedures in a group of 247 patients (97 allo-RIC and 150 conventional allo-SCT) is ongoing [36]. Moreover, many questions are still unanswered regarding this procedure: for example, the subgroups of patients who could experience a clearer benefit from it are still unknown, as are the best conditioning protocol and GVHD prophylaxis. The Spanish and British Cooperative Groups (fludarabine and melphalan at the same total doses but CsA plus MTX as GVHD prophylaxis or CsA plus alemtuzumab) have performed a comparative study including 129 patients with lymphoproliferative disorders, recipients of a sibling allogeneic transplantation and included in both prospective trials [37]. At last follow-up, there was no difference in disease status between groups. Alemtuzumab significantly reduced the incidence of both acute and chronic GVHD after transplantation but increased the percentage of CMV reactivation after transplantation. Patients treated with alemtuzumab often required DLIs to achieve similar disease control. A specific analysis that includes patients with HL from both prospective trials is under way [38].

Another issue of clinical interest with a potential influence on disease staging and the long-term outcome of these patients is the recent introduction of new functional imaging modalities, such as [^{18}F]fluorodeoxyglucose (FDG)–positron emission tomography (PET). Most previous studies, including this one, have defined response criteria both before transplantation and during follow-up on the basis of conventional radiographic characteristics [19,20,22]. The UK Cooperative Group has introduced for the first time FDG-PET or the combined strategy of FDG-PET

and conventional computed tomographic scanning in the allogeneic setting [23]. Advantages of this technique include the more precise evaluation not only of possible candidates for the procedure, but also of disease response during follow-up, as well as early identification of potential candidates for DLIs. Nevertheless, these benefits need to be proven prospectively in larger cohorts of patients.

In conclusion, allo-RIC is a feasible procedure for patients with relapsed or refractory HL, with an acceptable early and late TRM. Acute and chronic GVHD continue to be the major complications after transplantation and are responsible for a significant percentage of patient deaths after the allogeneic procedure. Nevertheless, the existence of responses after the infusion of donor lymphocytes, of durable responses after transplantation, and of a somewhat lower relapse rate in patients who develop extensive chronic GVHD indicates a clinically significant graft-versus-HL effect. OS seems promising, especially in chemosensitive patients with allo-RIC. Nevertheless, more patients included in prospective trials and longer follow-up are needed to really define the potential therapeutic role of allo-RIC in relapsed or refractory HL.

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