Dose-Adjusted EPOCH-Rituximab Combined With Fludarabine Provides an Effective Bridge to Reduced-Intensity Allogeneic Hematopoietic Stem-Cell Transplantation in Patients With Lymphoid Malignancies

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ABSTRACT

Purpose

There is currently no standard chemotherapy regimen for patients with lymphoid malignancies being considered for reduced-intensity conditioning allogeneic hematopoietic stem-cell transplantation (RIC-alloHSCT). The ideal regimen would provide disease control and result in lymphocyte depletion to facilitate engraftment. To this end, we developed a novel regimen by adding fludarabine to dose-adjusted continuous-infusion etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin plus with or without rituximab (DA-EPOCH-F/R).

Patients and Methods

One hundred forty-seven patients with lymphoid malignancy (median age, 50 years) who had heavily pretreated (median prior regimens, three) and chemo-refractory (47%) disease were treated with DA-EPOCH-F/R before RIC-alloHSCT. Patients received one to three consecutive cycles until achieving lymphocyte depletion ($CD4^+$ count $< 200/\mu L$) or progressive disease.

Results

Overall response rate was 41%; 39% of patients had stable disease. Toxicity included grade 4 neutropenia in 65% and thrombocytopenia in 25% of patients. DA-EPOCH-F/R resulted in lymphocyte depletion ($P < .001$), which was inversely associated with serum interleukin (IL) 7 and IL-15 levels. Of 147 patients, 143 patients proceeded to RIC-alloHSCT. Patients with lower $CD3^+$ ($P < .001$), $CD4^+$ ($P < .001$), and $CD8^+$ ($P < .001$) T-cell counts after DA-EPOCH-F/R were more likely to achieve full donor lymphoid chimerism by day +14 after transplant. Relative to nonresponders to DA-EPOCH-F/R, patients with complete and partial response had increased event-free survival (77.4 v 4.8 months; $P < .001$) and overall survival (98.5 v 16.2 months; $P < .001$).

Conclusion

DA-EPOCH-F/R safely provides tumor cytoreduction and lymphocyte depletion, thereby offering a bridge to RIC-alloHSCT in patients with aggressive lymphoid malignancies.

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INTRODUCTION

Allogeneic hematopoietic stem-cell transplantation (alloHSCT) using reduced-intensity conditioning (RIC) is a therapeutic option for patients with relapsed and refractory lymphoid malignancies, even after progression after autologous HSCT.¹⁻⁵ However, less intensive regimens yield reduced antitumor cytotoxicity and increased relapse compared with myeloablative regimens. Sensitivity to salvage chemotherapy and achievement of minimal residual disease before RIC-alloHSCT improve relapse-free and overall survival (OS).^{2,4-6} Despite the common

practice of administering pretransplant chemotherapy, no standard salvage regimen exists for relapsed or refractory lymphoid malignancy patients before RIC-alloHSCT.

The number of pretransplant regimens administered is associated with engraftment and full donor chimerism.⁷⁻⁹ This association may be related to variable lymphodepletion that occurs with the amount and type of chemotherapy.^{10,11} Post-transplant relapse inversely associates with degree of donor T-cell chimerism after RIC-alloHSCT. Specifically, full donor chimerism may enhance graft-versus-tumor effects.^{7,12} Therefore, desirable

pre-RIC transplant chemotherapy regimens would reduce both tumor burden and host lymphocytes.

We developed a pretransplant salvage regimen for patients with lymphoid malignancy who were candidates for RIC-alloHSCT. We used etoposide, vincristine, doxorubicin, cyclophosphamide, and prednisone (EPOCH) based on preclinical studies showing that *mdr-1*-expressing tumor cells were less resistant to prolonged chemotherapy exposure relative to bolus dosing.¹³ EPOCH, in which etoposide, vincristine, and doxorubicin are administered by continuous infusion, yielded high response rates in patients with relapsed and refractory non-Hodgkin's lymphoma.¹⁴ Because pharmacokinetic analyses demonstrated inter-patient variations in plasma etoposide and doxorubicin concentrations, follow-up studies incorporated individual patient dose adjustment based on hematopoietic nadir.¹⁵ Dose-adjusted (DA)-EPOCH was highly efficacious in patients with untreated large B-cell lymphomas.¹⁶ DA-EPOCH subsequently incorporated rituximab (DA-EPOCH-R), which was effective in patients with poor prognostic lymphoma variants and extensively pretreated patients.¹⁷⁻¹⁹

The immunosuppressive effect of fludarabine (F) is increased when used in combination with steroids, alkylators, or topoisomerase II inhibitors.²⁰ We reasoned that the combination of DA-EPOCH-R and fludarabine, which has activity against lymphoid malignancies, might induce significant host T-cell depletion.^{21,22} We assessed the safety, lymphodepleting capabilities, and clinical efficacy of DA-EPOCH-F/R in patients with lymphoid malignancies before RIC-alloHSCT.

PATIENTS AND METHODS

Eligibility Criteria

DA-EPOCH-F/R was administered on four sequential National Cancer Institute (NCI) protocols (NCT00019851, NCT00055744, NCT00077480, and NCT00074490) from January 2000 through December 2009. Rituximab was included starting with NCT0055744 in patients with CD20⁺ disease. All protocols were approved by the NCI institutional review board. Patients (18 to 75 years of age) with high-risk lymphoid malignancies, primary treatment failure, or relapse after autologous HSCT who had an HLA-matched first-degree relative or an 8/8 or 7/8 HLA-matched unrelated donor were eligible. Additional criteria included Eastern Cooperative Oncology Group performance status of 0 to 2, life expectancy more than 3 months, and adequate organ function. Patients were excluded for active infection, CNS involvement, or HIV or hepatitis B or C seropositivity. Patients received transplantation regardless of response to DA-EPOCH-F/R.

Chemotherapy and Dose Adjustments

In the initial patient cohort, cyclophosphamide and prednisone were decreased by 20%, and infusional etoposide, doxorubicin, and vincristine were reduced from 4 to 3 days to minimize potential adverse effects of combining EPOCH with fludarabine. After confirming safety, fludarabine was increased from 3 to 4 days, the infusional component of EPOCH was increased to 4 days, cyclophosphamide was increased from 600 to 750 mg/m², and prednisone was increased to 5 days. In the final iteration, DA-EPOCH-F consisted of fludarabine (25 mg/m² intravenously [IV], days 1 through 4), oral prednisone (60 mg/m², days 1 through 5), and cyclophosphamide (750 mg/m² IV, day 5); etoposide (60 mg/m²), doxorubicin (40 mg/m²), and vincristine (0.4 mg/m²) were given as a 24-hour continuous IV infusion (days 1 through 4; Table 1). Fludarabine was reduced by 20% in patients with creatinine clearance less than 70 mL/min. There was no upper limit on vincristine dose. Starting with the second cohort, rituximab was administered to patients with CD20⁺ disease (375 mg/m² IV, day 1 before fludarabine).

Table 1. DA-EPOCH-FR Regimen

Drug	Dose-Reduced Regimen	Final Regimen
Rituximab		375 mg/m ² IV* on day 1
Fludarabine	25 mg/m ² /d IV† days 1-3	25 mg/m ² /d IV† days 1-4
Infusional drugs‡		
Etoposide	50 mg/m ² days 1-3	50 mg/m ² days 1-4
Doxorubicin	10 mg/m ² days 1-3	10 mg/m ² days 1-4
Vincristine	0.5 mg/m ² days 1-3	0.4 mg/m ² days 1-4
Prednisone	60 mg/m ² PO days 1-4	60 mg/m ² /d PO days 1-5
Cyclophosphamide	600 mg/m ² IV§ on day 4	750 mg/m ² IV§ on day 5

Abbreviations: DA-EPOCH-F/R, dose-adjusted continuous-infusion etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin plus with or without rituximab; IV, intravenous; PO, orally.

*Rituximab was diluted to a concentration of 1 to 4 mg/mL in 0.9% NaCl injection (0.9% normal saline [NS]) and administered intravenously by standard rate titration as outlined in product labeling. Premedication included acetaminophen 650 mg PO and diphenhydramine 50 mg PO or IV given 1 hour before start of infusion.

†Fludarabine was diluted in 100 mL of 0.9% NS or 5% dextrose injection (D5W) for IV infusion over 30 minutes.

‡Etoposide, doxorubicin, and vincristine were combined together in a 3-in-1 admixture with dilution in 0.9% NS to produce a final etoposide concentration < 250 µg/mL in polyolefin-lined infusion bags.²³

§Cyclophosphamide was diluted in 100 mL 0.9% NS or D5W for intravenous infusion over 15 to 30 minutes. Intravenous hydration (500 mL of 0.9% NS) was given over 1 to 2 hours before and after cyclophosphamide administration. A 5-HT₃ receptor antagonist antiemetic was given before administration of cyclophosphamide and for usually 2 to 3 days after.

Patients received 96-hour infusions via central venous access using portable infusion pumps. A 24-hour solution containing vincristine, doxorubicin, and etoposide mixed in 0.9% normal saline was prepared daily.²³ Dose adjustment consisted of 20% escalation in etoposide, doxorubicin, and cyclophosphamide with each successive cycle, providing the prior cycle did not result in febrile neutropenia or prolonged neutropenia (two consecutive biweekly absolute neutrophil count [ANC] values < 500 cells/µL).

All patients received filgrastim (5 µg/kg/d) by subcutaneous injection from day 6 until ANC more than 1,000/µL × 2 three days apart or more than 5,000/µL once after nadir. DA-EPOCH-F/R was administered for one to three cycles until the patient had CD4⁺ count less than 50 to 200/µL (variable per protocol) or progressive disease. Cycles were repeated every 21 days, provided adequate hematologic recovery (ANC > 500/µL; platelet count > 50,000/µL). Patients received infectious disease prophylaxis with acyclovir, trimethoprim/sulfamethoxazole, and fluconazole.

RIC-AlloHSCT

RIC consisted of fludarabine (30 mg/m² IV) and cyclophosphamide (1,200 or 300 mg/m²/d IV, day -6 to -3; dose protocol dependent). Graft-versus-host disease (GVHD) prophylaxis varied by protocol and comprised cyclosporine alone or in combination with sirolimus or methotrexate and/or Th2 cells.²⁴

Lymphocyte Monitoring by Flow Cytometry

Peripheral blood was collected before treatment and between days 18 through 20 of each cycle. Flow cytometry was performed using whole-blood lysis in a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory (Science Applications International, Frederick, MD). Absolute numbers of CD3⁺, CD4⁺, and CD8⁺ T cells and B and natural killer (NK) cells per liter of blood were calculated on the basis of percentage in the lymphocyte gate, percentage of lymphocytes (defined as CD45^{bright}, CD14⁻, low side scatter), and WBC counts (Coulter, Miami, FL). CD4⁺ and CD8⁺ T cells were defined as CD3⁺, CD4⁺, or CD3⁺ CD8⁺, respectively. B cells were defined as CD3⁻ CD19⁺; NK cells were defined as CD3⁺ CD56⁺.

Serum Interleukin-7 and Interleukin-15

Interleukin-7 (IL-7) and interleukin-15 (IL-15) are T-cell homeostatic cytokines that vary with lymphocyte depletion.^{25,26} In a subset of patients,

serum was obtained before treatment and after hematopoietic recovery from each cycle for IL-7 and IL-15 determination. Serum samples were frozen at -80°C until analysis. Before analysis, samples were thawed and analyzed by high-sensitivity colorimetric enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN). Standard curves were prepared using serial dilutions. Plates were read with microplate reader (Molecular Devices, Sunnyvale, CA) and analyzed using SOFTmax Pro 3.0 software (Molecular Devices). Intra- and inter-assay variability was less than 15%. The IL-15 QuantiGlo enzyme-linked immunosorbent assay (R&D Systems) was used to measure IL-15 levels in cryopreserved heparinized plasma, collected concurrent with blood assayed for lymphocyte populations.

Statistical Analysis

Primary end points were response rates, toxicities, and degree of lymphocyte depletion. Secondary end points were engraftment, response rates, and survival. Data on demographics, response, immune depletion, chimerism, and toxicity (National Cancer Institute Common Toxicity Criteria version 3.0) were analyzed using descriptive statistics. Changes in $\text{CD}3^{+}$, $\text{CD}8^{+}$, and $\text{CD}4^{+}$ were reported using relative (percentage) change compared with pretreatment or an earlier cycle. Significance from zero was determined by Wilcoxon signed rank test.

Differences between groups for continuous parameters were determined using an exact form of the Wilcoxon rank sum test. A Cochran-Armitage test for trend was used to determine the association between ordered categorical parameters and dichotomous parameters. A Jonckheere-Terpstra test for trend was used to determine the association between two ordered categorical parameters or between a continuous parameter and an ordered categorical parameter. Survival was determined from date of stem-cell infusion to date of death or last follow-up; event-free survival (EFS) was determined from date of stem-cell infusion to date of progression, death, or last follow-up. The Kaplan-Meier method was used to determine probability of survival and EFS as a function of time. A log-rank test was used to determine significance of differences among Kaplan-Meier curves for survival or EFS. EFS and OS were each examined with respect to day +14 $\text{CD}3^{+}$ chimerism, $\text{CD}4^{+}$ count decline and after DA-EPOCH-F/R, and NK baseline. Analyses showing fraction of decline in immune reconstitution parameters by cycle of DA-EPOCH-F/R were performed using formulas in which CX represents a given parameter at the stated cycle, for example: $\text{C}2\text{-C}1/\text{C}2\text{-C}0$, $\text{C}1\text{-C}0/\text{C}2\text{-C}0$ for patients who received two cycles of DA-EPOCH-F/R. All *P* values are two-tailed and have not been adjusted for multiple comparisons.

RESULTS

Patient Demographics

One hundred forty-seven patients, median age of 50 years (range, 21 to 71 years), with high-risk lymphoid malignancies received treatment with DA-EPOCH-F/R (Table 2). Patients had received a median of three prior regimens (range, one to 13 regimens), including anthracyclines (91%), fludarabine (36%), platinum (37%), rituximab (70%), and autologous HSCT (24%). Twenty-seven percent of patients had disease that was refractory to anthracycline-based regimens, 39% had disease that was refractory to platinum-based regimens, and 39% had disease that was refractory to cytarabine-based regimens. Forty-seven percent of patients had disease that was refractory to their most recent chemotherapy regimen. Patients received a median of two cycles of DA-EPOCH-F/R.

Lymphocyte-Depleting Effects of DA-EPOCH-F/R

DA-EPOCH-F/R significantly depleted lymphocytes (Table 3; $P < .001$ for changes in baseline from each cycle for $\text{CD}3^{+}$, $\text{CD}4^{+}$, and $\text{CD}8^{+}$ counts). $\text{CD}3^{+}$ counts decreased from a median of $574/\mu\text{L}$ (range, 5 to 4,833) to $179/\mu\text{L}$ (range, 1 to 1,557), $\text{CD}4^{+}$ counts de-

Table 2. Patient Characteristics

Characteristic	No. of Patients
No. of patients	147
Age, years	
Median	50
Range	21-71
Sex	
Male	95
Female	52
Histologic findings	
Diffuse large B-cell lymphoma	44
Mantle cell	18
Follicular NHL	19
Hodgkin's lymphoma	23
CLL	19
NK/PTCL	15
Other	9
No. of prior regimens	
Median	3
Range	1-13
Prior autologous stem-cell transplant	36
Prior anthracycline	134
Prior fludarabine	53
Prior platinum	53
Prior rituximab	102
Disease status at enrollment	
Chemosensitive	76
Chemoresistant	67
Unknown	4

Abbreviations: CLL, chronic lymphocytic leukemia; NHL, non-Hodgkin's lymphoma; NK, natural killer; PTCL, peripheral T-cell lymphoma.

creased from a median of $244/\mu\text{L}$ (range, 5 to 3,888) to $96/\mu\text{L}$ (range, 0 to 1,332), and $\text{CD}8^{+}$ counts decreased from a median of $249/\mu\text{L}$ (range, 1 to 3,478) to $65/\mu\text{L}$ (range, 1 to 1,195), with a corresponding median relative decrease based on paired changes within each patient of 68%, 65%, and 73% for $\text{CD}3^{+}$, $\text{CD}4^{+}$, and $\text{CD}8^{+}$, respectively.

Data were also evaluated according to the decrease attributable to each cycle (Table 3). Among patients receiving two DA-EPOCH-F/R cycles, the mean decrease in T-cell numbers attributable to the first versus second cycle was 67% versus 33% ($\text{CD}3^{+}$), 41% versus 59% ($\text{CD}4^{+}$), and 70% versus 30% ($\text{CD}8^{+}$). Among patients receiving three DA-EPOCH-F/R cycles, the total decrease in T-cell numbers attributable to the first versus second versus third cycle was 54% versus 19% versus 27% ($\text{CD}3^{+}$), 41% versus 38% versus 21% ($\text{CD}4^{+}$), and 67% versus 3% versus 30% ($\text{CD}8^{+}$). Lymphodepletion was significantly greater in patients who received a 25% dose increase in DA-EPOCH-F/R only if they received three cycles ($P < .05$ for each of $\text{CD}3^{+}$, $\text{CD}4^{+}$, and $\text{CD}8^{+}$ cell counts).

Serum IL-7 and IL-15

Twenty-six patients had IL-7 and IL-15 tested at baseline and after DA-EPOCH-F/R cycles. Median IL-7 levels ($n = 22$) increased by a median of 5.6 pg/mL after 1 cycle and 7.5 pg/mL after 1 to 3 cycles (Table 4). Median IL-15 levels ($n = 20$) increased by a median of 3.6 pg/mL after one cycle and 4.7 pg/mL after one to three cycles. Increase in IL-7 was associated with weak to moderate decrease in $\text{CD}3^{+}$ ($r = -0.37$; $P = .07$), $\text{CD}4^{+}$ ($r = -0.34$; $P = .1$), and $\text{CD}8^{+}$

Table 3. Lymphocyte Depletion After DA-EPOCH-F/R by Cycle and Percentage Decrease by Cycle

Patient Group	Baseline	T-Cell Numbers/ μ L				Decrease			
		Post C1	Post C2	Post C3	Post Total Cycles	Post C1	Post C2	Post C3	Post Total Cycles
All patients (n = 147)									
CD3 ⁺	574	291	218	198	179				68
CD4 ⁺	244	161	130	110	96				65
CD8 ⁺	249	106	69	87	65				73
Patients receiving 2 cycles (n = 31)									
CD3 ⁺	673	280	151			67	33		
CD4 ⁺	251	138	75			41	59		
CD8 ⁺	312	124	63			70	30		
Patients receiving 3 cycles (n = 45)									
CD3 ⁺	954	456	296	198		54	19	27	
CD4 ⁺	429	300	176	110		41	38	21	
CD8 ⁺	363	135	78	87		67	3	30	

Abbreviation: C, cycle; DA-EPOCH-F/R, dose-adjusted continuous-infusion etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin plus with or without rituximab.

($r = -0.38$; $P = .06$) counts, respectively. Increase in IL-15 was moderately well associated with decrease in CD3⁺ ($r = -0.56$; $P = .004$), CD8⁺ ($r = -0.49$; $P = .015$), and NK cell ($r = -0.48$; $P = .018$) counts, respectively.

Response to DA-EPOCH-F/R

Response was determined by comparing tumor measurements after DA-EPOCH-F/R to those obtained at enrollment. DA-EPOCH-F/R yielded an overall response rate (ORR) of 41% (complete response [CR] = 16%, partial response [PR] = 25%; Table 5); an additional 39% had stable disease (SD). Sixty-two percent of patients with chemotherapy-sensitive disease, defined as CR or PR to their most recent prior regimen, experienced a response (CR = 25%, PR = 37%) to DA-EPOCH-F/R as compared with 17% (CR = 5%, PR = 12%) in

patients with chemotherapy-resistant disease ($P < .001$). Among patients determined to have SD after DA-EPOCH-F/R, 29% had a PR, 29% had SD, and 25% had progressive disease (PD) to their last pre-enrollment therapy. Response also varied by histology ($P = .047$), with 57% ORR in indolent non-Hodgkin's lymphoma (CR = 14%, PR = 43%), 37% in aggressive non-Hodgkin's lymphoma (CR = 21%, PR = 16%), 43% in Hodgkin's lymphoma (CR = 14%, PR = 29%), 13% in T-cell lymphoma (CR = 7% PR = 7%), and 58% in chronic lymphocytic leukemia (CR = 11%, PR = 47%). Patients with prior anthracycline exposure had a 40% ORR, whereas those without had a 58% ORR ($P = .23$; Fisher's exact test). Patients with prior platinum exposure had a 35% ORR, whereas those without had a 44% ORR ($P = .28$). Number of cycles of DA-EPOCH-F/R was significantly associated with ORR: one cycle, 17%; two cycles, 33%; three

Table 4. Relationship Between IL-7 and IL-15 Levels After DA-EPOCH-F/R and T-Cell Depletion

	No.	Lower 25%	Median	Upper 25%	<i>P</i>	<i>r</i> Versus IL-7	<i>P</i> Versus IL-7	<i>r</i> Versus IL-15	<i>P</i> Versus IL-15
CD3 ⁺									
Post EPOCH minus baseline*	24	-937	-408	-35	< .001	-0.37	.07	-0.56	.004
Cycle 1 minus baseline†	25	-538	-398	-68	< .001				
CD4 ⁺									
Post EPOCH minus baseline	24	-481	-150	-58	< .001	-0.34	.10		
Cycle 1 minus baseline	25	-247	-80	-22	< .001				
CD8 ⁺									
Post EPOCH minus baseline	24	-564	-202	-20	< .001	-0.38	.06	-0.49	.015
Cycle 1 minus baseline	25	-423	-164	-36	< .001				
NK									
Post EPOCH minus baseline	24	-18	34	224	.009			-0.48	.018
Cycle 1 minus baseline	25	-46	4	108	.20				
IL-7									
Post EPOCH minus baseline	22	0.6	7.5	16.9	< .001				
Cycle 1 minus baseline	22	0.4	5.6	18.6	.003				
IL-15									
Post EPOCH minus baseline	20	2.6	4.7	7.5	< .001				
Cycle 1 minus baseline	18	0.8	3.6	7.4	< .001				

Abbreviations: DA-EPOCH-F/R, dose-adjusted continuous-infusion etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin plus with or without rituximab; IL, interleukin; NK, natural killer.

*After total cycles of DA-EPOCH-F/R received (one to three).

†After one cycle of DA-EPOCH-F/R.

Table 5. Response to DA-EPOCH F/R by Histology, Chemosensitivity, and Number of Cycles

Variable	CR + PR		SD + PD	
	No.	%	No.	%
Histology				
DLBCL	13	30	30	70
Mantle cell lymphoma	9	60	6	40
Follicular lymphoma	12	71	5	29
Hodgkin's lymphoma	9	41	13	59
CLL/SLL	12	63	7	37
T-cell lymphomas	2	13	13	87
Chemosensitivity				
Chemosensitive	44	62	27	38
Chemoresistant	11	17	53	83
No. of DA-EPOCH-F/R cycles				
1	11	17	54	83
2	10	33	20	67
3	36	86	7	17

Abbreviations: CLL, chronic lymphocytic leukemia; CR, complete response; DA-EPOCH-F/R, dose-adjusted continuous-infusion etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin plus with or without rituximab; DLBCL, diffuse large B-cell lymphoma; PD, progressive disease; PR, partial response; SD, stable disease; SLL, small lymphocytic lymphoma.

cycles, 84%; $P < .001$). ORR was not different in patients who received the final versus dose-reduced EPOCH-F/R regimen ($P > .10$).

Toxicity of DA-EPOCH-F/R

DA-EPOCH-F/R was well tolerated. The most common toxicities were hematologic. Sixty-five percent of patients experienced grade 4 neutropenia, 30% developed neutropenic fever, and 25% had grade 4 thrombocytopenia. The incidence and severity of neutropenia was expected, as the treatment was dose adjusted to achieve a nadir ANC less than $500/\mu\text{L}$. Grade 3 gastrointestinal, neurologic, and cardiac toxicities occurred in 7%, 2%, and 4% of patients, respectively. Grade 4 nonhematologic toxicity was seen in 6% of patients. Grade 3 infections were noted in 10% of cycles. One patient experienced grade 4 infection, and one patient died as a result of sepsis. There was no increase in toxicity in patients with prior autologous transplant (data not shown).

Transplantation Results

Of 147 patients, 143 proceeded to HSCT (129 related, 14 unrelated; one patient withdrew consent, one had grade 4 toxicity, and two had PD). There were no graft rejections. Sixty-two (49%) of 127 evaluable patients achieved more than 95% donor chimerism by day +14 after HSCT; 16 patients were not evaluable because of insufficient sample. Patients with lower lymphocyte counts after DA-EPOCH-F/R ($\text{CD}3^+$, $P < .001$; $\text{CD}4^+$, $P < .001$; and $\text{CD}8^+$, $P < .001$) were more likely to achieve full donor chimerism by day +14 after transplant. Grade 2 to 4 acute GVHD was seen in 50 (38%) of 132 evaluable patients. Of 112 evaluable patients, chronic GVHD was observed in 65%.

Median EFS was 12.3 months (77.4 months ν 4.8 months for DA-EPOCH-F/R responders and nonresponders, respectively). Median OS was 65.7 months (Fig 1A). Median survival assessed according to pretransplant response to DA-EPOCH-F/R revealed 98.5 months for responders versus 16.2 months for nonresponders

Fig 1. (A) Overall survival for all patients enrolled onto study. (B) Comparison of overall survival in patients who achieved complete (CR) or partial response (PR) versus stable (SD) or progressive disease (PD) after dose-adjusted continuous-infusion etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin plus with or without rituximab (DA-EPOCH-F/R). (C) Comparison of overall survival in patients who achieved CR or PR versus SD or PD by response to DA-EPOCH-F/R.

($P < .001$; Fig 1B). Each of the three OS curves for patients with DA-EPOCH-F/R response greater than PD was significantly greater than those with PD (global $P < .001$; $P < .001$ for each comparison individually; Fig 1C). Median 2-year OS and EFS determined by histology revealed follicular (64.2% and 64.3%, respectively), diffuse large B-cell lymphoma (42.4% and 27.5%), Hodgkin's lymphoma

(53.4% and not reached), T-cell lymphoma (61.7% and 46.3%), chronic lymphocytic leukemia (81.1% and 55.9%), and mantle-cell lymphoma (63.1% and 45.8%). Of the 56 responders, 13 (23.2%) had a relapse event; by comparison, in nonresponders, 42 (53.8%) of 78 patients had a relapse event. Achievement of full donor CD3⁺ chimerism by day +14 was inversely associated with OS ($P = .017$) but not EFS; median survival was not met in patients with less than 95% chimerism at day +14, whereas patients with $\geq 95\%$ chimerism at day +14 had a median survival of 20.6 months. EFS and OS were not associated with CD4⁺ count after DA-EPOCH-F/R, NK baseline, or decline in CD4⁺ count.

DISCUSSION

RIC regimens for patients with relapsed or refractory lymphoid malignancies increase the reliance on graft-versus-tumor effects for the curative potential of alloHSCT.^{27,28} RIC-alloHSCT has been historically of limited benefit for refractory lymphoid malignancies, and response to chemotherapy has been identified in retrospective analyses as a prognostic factor for outcomes after RIC-alloHSCT.^{6,29-32} It therefore becomes important to use effective pretransplant salvage regimens for patients with lymphoid malignancies before reduced-intensity transplantation.

We developed DA-EPOCH-F/R as a pretransplant salvage regimen for patients with aggressive lymphoid malignancies. It was our intent to design a regimen that provided disease control and host lymphocyte depletion. This latter criterion was based on the significant variability of patients' pretransplant immune T-cell numbers and the role of T cells in resisting allogeneic engraftment. We hypothesized that depletion of host T cells before transplantation would result in greater likelihood of rapid and complete engraftment of allogeneic stem cells, which is not consistently achieved with many RIC regimens, especially if alternative donor stem cells (eg, HLA-matched unrelated donors) and/or T-cell depleted allografts are used.^{5,6}

DA-EPOCH-F/R reduced circulating T-cell numbers by 65% to 75% and reduced the variability in pretransplant host immune status. This reduction in T-cell numbers was associated with an increase in serum IL-7 and IL-15. Because IL-7 and IL-15 are homeostatic cytokines that increase during total body immune depletion, reductions in circulating T-cell numbers may reflect total-body T-cell depletion.²⁴ Therefore, circulating T-cell numbers or serum IL-7/IL-15 levels might be used to monitor host T-cell depletion. Several studies have demonstrated that lymphocyte-depleting chemotherapy before non-myeloablative transplantation may be required to consistently achieve early post-transplant full donor chimerism.^{11,33,34} The results of the current study confirm our hypothesis in a larger cohort.

In this heavily pretreated patient population, DA-EPOCH-F/R provided disease control in most patients with predictable and manageable toxicities. Clinical responses were seen in 41% of DA-EPOCH-F/R recipients, many of whom had previously received anthracycline-based or platinum-containing therapy; 47% of patients were determined to have chemotherapy-refractory disease immediately before study entry. The increase in response with each additional cycle, although encouraging, may be due in part to selection bias. It is important to note that the majority of patients (80%) maintained at

least disease stability, with only 27 of 138 evaluable patients having disease progression during DA-EPOCH-F/R. Among patients whose disease remained stable while receiving DA-EPOCH-F/R, approximately 50% achieved long-term OS after RIC-alloHSCT. Patients with SD are often classified as having "chemotherapy-refractory" disease and may be excluded from transplant trials. In this study, we have demonstrated that such patients may respond to continuous-infusion DA-EPOCH-F/R and derive benefit from transplantation.^{6,35} It is important to note that OS in this analysis was lower in patients who achieved early CD3⁺ full donor chimerism. It is difficult to ascertain a potential mechanism to account for this because multiple transplant approaches were used after DA-EPOCH-F/R therapy (different donor types, presence or absence of donor Th2 cell infusion, varied immunosuppression regimens). Further analyses would be required to assess whether this association might be due to increased infection and/or increased GVHD.

Physicians who manage patients with lymphoid malignancies often struggle to choose an appropriate regimen for patients with PD before allogeneic transplant or for patients awaiting matched unrelated donor transplant. When choosing a pretransplant regimen, the main goal is to achieve at least disease stability, if not disease response. DA-EPOCH-F/R succeeds in providing disease control in heavily pretreated patients with relapsed and refractory disease without excessive toxicity and results in host T-cell depletion, promoting donor engraftment in the post-transplant setting.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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