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Infused Autograft Lymphocyte to Monocyte Ratio and Survival in Diffuse Large B Cell Lymphoma

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Infused autograft absolute lymphocyte count is a prognostic factor for survival after autologous peripheral hematopoietic stem cell transplantation (APHSCT) for diffuse large B cell lymphoma (DLBCL). $CD14^+$ $HLA\text{-}DR^{low/neg}$ immunosuppressive monocytes affect tumor growth by suppressing host antitumor immunity. Thus, we set out to investigate if the infused autograft lymphocyte to monocyte ratio (A-LMR), as a biomarker of host immunity (ie, lymphocytes) and immunosuppression (ie, monocytes), affects survival after APHSCT. From 1994 to 2012, 379 DLBCL patients who underwent APHSCT were studied. The 379 patients were randomly divided into a training set (n = 253) and a validation set (n = 126). Receiver operating characteristic and area under the curve identified an A-LMR ≥1 as the best cut-off value, which was validated by the k-fold cross-validation in the training set. Multivariate analysis showed A-LMR to be an independent prognostic factor for survival in the training set. Patients with an A-LMR ≥ 1.0 experienced superior overall survival (OS) compared with patients with an A-LMR <1.0 (median OS: 167.2 versus 17.6 months; 5-year OS: 73% [95% confidence interval (CI), 63% to 80%] versus 30% [95% CI, 2% to 38%], $P < .0001$, respectively) in the training set. In the validation set, an A-LMR ≥ 1 showed a median OS of 181.2 months versus 19.5 months for an A-LMR <1, and 5-year OS rates of 67% (95% CI, 52% to 79%) versus 35% (95% CI, 25% to 47%), $P < .0001$, respectively. The A-LMR provides a platform to engineer immunocompetent autograft to improve clinical outcomes in DLBCL patients undergoing APHSCT.

INTRODUCTION

Infused autograft absolute lymphocyte count (A-ALC) $\geq .5 \times 10^9$ cells/kg is a prognostic factor for survival after autologous peripheral hematopoietic stem cell transplantation (APHSCT) for lymphoma [1] and multiple myeloma [2,3]. However, some patients relapse after APHSCT, despite infusing an A-ALC $\geq .5 \times 10^9$ cells/kg, whereas other patients with an A-ALC $< .5 \times 10^9$ cells/kg remain in complete remission after APHSCT. Recent gene-expression profiling studies in non-Hodgkin lymphoma have demonstrated that gene expression by tumor-infiltrating myeloid-derived suppressor cells predicts clinical outcomes [4,5]. Furthermore, monocyte-derived cells may also provide trophic factors, which directly promote the growth and survival of malignant lymphocytes [6,7].

Two main subsets of myeloid-derived suppressor cells (MDSCs) have been proposed in humans: the granulocytic and the monocytic MDSCs [8]. The human monocytic MDSCs subset has been characterized as monocytic $CD14^+$ cells with a low level or lack of the antigen-presenting HLA-DR molecules ($CD14^+HLA\text{-}DR^{low/neg}$ cells) [8]. Our group reported the presence of circulating immunosuppressive $CD14^+HLA\text{-}DR^{low/neg}$ peripheral blood monocytes in patients with lymphoma [9]. These circulating $CD14^+HLA\text{-}DR^{low/neg}$ monocytes are recruited and transformed into tumor-associated macrophages by the tumor, affecting survival in cancer patients [10]. Stem cell mobilization agents (ie, Neupogen [Amgen, Inc., Thousand Oaks, CA]) have been implicated in the mobilization of monocytes, in addition to stem cells in normal volunteers [11], as well as in patients undergoing APHSCT [12]. Thus, we studied the impact of the autograft absolute lymphocyte to monocyte ratio (A-LMR) as a surrogate biomarker combining host immunity (ie, A-ALC) and immunosuppression (ie, autograft absolute monocyte count [A-AMC]), on clinical outcomes after APHSCT in patients with diffuse large B cell lymphoma (DLBCL).

MATERIALS AND METHODS

Patient Population

To participate in the study, patients were required to be candidates for APHSCT with the diagnosis of de novo DLBCL, have chemosensitive disease

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before APHSCT, and have mobilized enough peripheral blood stem cells to proceed with APHSCT (minimum of 2.0×10^6 CD34 cells/kg). Patients were excluded if they failed to mobilize stem cells, required bone marrow harvest, were infused with both peripheral blood and bone marrow harvest–derived stem cells, participated in stem cell transplantation clinical trials, or had either concomitant lymphoma histologies at diagnosis or transformed lymphomas. No patients were lost to follow-up. From 1994 to 2012, 379 DLBCL patients qualified for the study. All patients gave written, informed consent allowing the use of their medical records for medical research. Approval for the retrospective review of these records was obtained from the Mayo Clinic institutional review board and was in accordance with federal regulations and the Declaration of Helsinki.

Endpoints

The primary endpoint of the study was to assess the impact of A-LMR on overall survival (OS), progression-free survival (PFS), and lymphoma-specific survival (LSS) from the time of APHSCT. The second endpoint was to identify if A-ALC, A-AMC, and A-LMR directly affect the recovery of day 15 absolute lymphocyte count (ALC-15), day 15 absolute monocyte count (AMC-15), and day 15 absolute lymphocyte to monocyte ratio (LMR-15) after APSHCT. The ALC-15, AMC-15, and LMR-15 were calculated from the complete blood cell count [13] obtained at day 15 after APHSCT. The LMR-15 ratio was calculated by dividing ALC-15 over AMC-15. The infused A-ALC for each apheresed unit collection was calculated as follows: A-ALC = % collection lymphocytes × (absolute WBC/kg). In similar fashion, the infused A-AMC for each apheresed unit collection was calculated as follows: A-AMC = % collection monocytes × (absolute WBC/kg). The A-LMR was then calculated by dividing the A-ALC by the A-AMC.

Prognostic Factors

The following prognostic factors were evaluated in the study: international prognostic index (IPI) [14] at diagnosis, age at diagnosis (≥60 versus <60 years), lactate dehydrogenase (LDH) at diagnosis (>normal), performance status at diagnosis (>1 versus ≤1), extranodal sites at diagnosis (>1 versus ≤1), and stage at diagnosis (III/IV versus I/II), IPI at relapse [15], age at relapse (>60 versus ≤60 years), extranodal sites at relapse (>1 versus ≤1), LDH at relapse (abnormal), performance status at relapse (>1 versus ≤1), and stage at relapse (I/II versus III/IV), disease status before APHSCT (complete remission [CR] versus partial response), A-ALC, A-AMC, A-LMR, ALC-15, AMC-15, LMR-15, and pretransplantation rituximab versus no rituximab [16].

Peripheral Blood Stem Cell (Autograft) Collections

The 3 different types of instruments used at our facility during the period examined in this study were the COBE Spectra (Gambro BCT, Lakewood, CO), Baxter Amicus (Baxter Healthcare, Deerfield, IL), and Fenwal CS3000 (Baxter). All patients were collected using a single instrument type, based on availability of the instrument on the day of collection. Patients received granulocyte colony–stimulating factor (G-CSF) for mobilization at a dose of 10 μg/kg daily for 5 to 7 consecutive days by subcutaneous injection. After the peripheral blood $CD34^+$ cell count was ≥ 10 cells/μL on G-CSF, patients began daily apheresis until a minimum target of 2.0×10^6 CD34 cells/kg was reached. If the peripheral blood CD34 on day 4 on G-CSF was less than 10 cells/μL, the addition of plerixafor (Sanofi-Aventis, Bridgewater, NJ) .24 mg/kg was allowed.

Conditioning Regimens

Conditioning regimens were as follows: 326 patients received BEAM (BCNU [300 mg/m^2] on day −6, etoposide [100 mg/m^2] twice daily from days −5 to −2, ARA-C [cytarabine] [100 mg/m^2] twice daily from days −5 to −2, and melphalan (140 mg/m^2) on day −1); 47 patients received BEAC (BCNU [300 mg/m^2] on day −6, etoposide [100 mg/m^2] twice daily from days −5 to −2, ARA-C [100 mg/m^2] twice daily from days −5 to −2, and cyclophosphamide (35 mg/kg) on day −1); and 6 patients received cyclophosphamide (60 mg/m^2 × 2) and total body irradiation (12 Gy).

Response and Survival

Response criteria were based on the guidelines from the International Harmonization Project on Lymphoma [17]. OS was measured from the date of transplantation to the date of death or last follow-up. *PFS* was defined as the time from transplantation to the time of progression, relapse, death, or last follow-up. *LSS* was defined as the time from transplantation to death as a result of lymphoma.

Statistical Analysis

To assess the validity of the A-LMR as a prognostic factor for APHSCT in DLBCL, patients were randomly divided into 2 groups. Two-thirds were assigned to the training set (n = 253) to develop the best cut-off value for A-LMR, and the remaining one-third were assigned to the validation set (n = 126) to assess the prognostic ability of A-LMR. OS, PFS, and LSS were analyzed using the approach of Kaplan and Meier [18]. Differences between survival curves were tested for statistical significance using the 2-tailed log-rank test. The Cox proportional hazard model [19] was used for the univariate and multivariate analysis to evaluate the variables under the prognostic factors section to assess their impact on post-APSCHT OS, PFS, and LSS times. The choice of optimal cut-off for A-AMC, A-LMR, AMC-15, and LMR-15 to assess survival was based on their utility as markers for the clinically relevant binary outcome of death/survival, using the receiver operating characteristics curves (ROC) and area under the curve (AUC). The binary clinical outcome (death/survival) was established at 5 years after APHSCT. Patients were classified as "alive/censored" when follow-up time was greater than 5 years and "death" for patients known to have died before this time point [20]. A k-fold cross-validation with k values of 10 was performed to validate the results of A-AMC, A-LMR, AMC-15, and LMR-15. Randomly chosen subsets containing 90% of the cohort were used for training, and the remaining 10% were left for testing. The cross-validation process was then repeated 10 times. Based on this analysis, cross-validation AUC by the ROC were produced, representing the discriminating accuracy of A-AMC, A-LMR, AMC-15, and LMR-15 for the binary clinical outcomes of death/survival. The cut-offs of A-ALC ≥$.5 \times 10^9$ cells/kg and ALC-15 ≥500 cells/μL used in this study were based on our previous publications [1,21].

Chi-square tests and Fisher exact tests were used to determine relationships between categorical variables as appropriate. The Wilcoxon rank test was used to determine associations between continuous variables and categories, and nonparametric tests were used to evaluate associations for continuous variables. All *P* values represented were 2-sided and statistical significance was declared at $P < .05$.

RESULTS

Patients Characteristics

The median age at the time of transplantation for this cohort of 379 DLBCL patients was 58 years (range, 17 to 78 years). The distribution of additional baseline characteristics for these patients is presented in Table 1, based on the training set and validation set. The median follow-up for the entire cohort was 27.1 months (range, 1 to 238.8 months) and for the living patients (n = 181), 77.4 months (range, 2.3 to 238.8 months). The day 100 transplantation-related mortality for the cohort was 2% (8 of 379). One hundred and fifty-nine patients died due to relapse/progression of lymphoma. Thirty-one patients died of unrelated lymphoma causes, excluding the 8 patients who died in the first 100 days after APHSCT.

Cut-off Values for ALC-15, AMC-15, and ALC-15 to AMC-15 Ratio for Survival Analysis

ROC curves and AUC were used to determine the optimal cut-off points for A-LMR, A-AMC, AMC-15, and LMR-15, based on their utility as markers for the clinical binary outcome of death/survival in the training set. The A-LMR ≥1 had an AUC of .73 (95% confidence interval [CI], .55 to .91) with a sensitivity of 73% (95% CI, 68% to 78%) and specificity of 68% (95% CI, 62% to 74%), $P < .04$ (Figure 1A). The A-AMC <$.5 \times 10^9$ cells/kg had an AUC of .63 (95% CI, .57 to .69) with a sensitivity of 63% (95% CI, 57% to 69%) and specificity of 70% (95% CI, 64% to 76%), $P < .04$. The AMC-15 ≤ 600 cells/μL had an AUC of .66 (95% CI, .61 to .74) with a sensitivity of 71% (95% CI, 65% to 77%) and specificity of 67% (95% CI, 54% to 66%), $P < .0001$. The LMR-15 ≥ 1 had an AUC of .84 (95% CI, .81 to .87) with a sensitivity of 80% (95% CI, 75% to 85%) and a specificity of 79% (95% CI, 74% to 83%), $P < .0001$. An internal validation of A-LMR, A-AMC, AMC-15, and LMR-15 performances as markers for the clinical binary outcomes of death/survival was performed using k-fold cross-validation with k = 10. We obtained an average AUC of .73 (95% CI, .67 to .80) over the 10 validation sets for A-LMR with a standard deviation of ± .02. In similar fashion, we obtained an average AUC for A-AMC of .62 (95% CI, .56 to .68); for AMC-15, of .67 (95% CI, .59 to .75); and for LMR-15, of .83 (95% CI, .78 to .89). We report the ROC

Table 1
Baseline Characteristics in the Training Set and Validation Set

Variable	Training Set n = 253			Validation Set n = 126			*P* Value*
	A-LMR ≥ 1 (n = 126)	A-LMR < 1 (n = 127)	*P* Value	A-LMR ≥ 1 (n = 52)	A-LMR < 1 (n = 74)	*P* Value	
At diagnosis							
Age, median (range), yr	58 (19-72)	5 (23-76)	.70	52 (21-76)	60 (17-74)	.20	<.01
Gender			.80			.50	.50
Male	78 (62%)	77 (61%)		36 (69%)	46 (62%)		
Female	48 (38%)	50 (39%)		16 (31%)	28 (38%)		
Extra-nodal			.70			.30	.50
0	73 (58%)	76 (60%)		34 (65%)	46 (62%)		
1	38 (30%)	33 (26%)		11 (21%)	15 (20%)		
2	13 (10%)	14 (11%)		7 (14%)	10 (14%)		
3	2 (2%)	3 (2%)		0 (0%)	3 (4%)		
4	0 (0%)	1 (1%)					
LDH, median (range), U/L	211 (80-2400)	281 (60-2250)	.20	300 (151-1460)	264 (139-1812)	.20	.20
PS			.10			.10	.20
0	24 (19%)	25 (20%)		13 (25%)	14 (19%)		
1	101 (80%)	96 (75%)		37 (71%)	57 (77%)		
2	1 (1%)	6 (5%)		0 (0%)	3 (4%)		
3				2 (4%)	0 (0%)		
Stage			.70			.50	<.04
I	13 (10%)	18 (14%)		1 (2%)	5 (7%)		
II	21 (17%)	21 (16%)		5 (10%)	10 (13%)		
III	24 (19%)	25 (20%)		12 (23%)	17 (23%)		
IV	68 (54%)	63 (50%)		34 (65%)	42 (57%)		
IPI risk factors							
Age, yr			.70			.90	<.01
>60	53 (42%)	56 (44%)		15 (29%)	23 (30%)		
≤60	73 (58%)	71 (56%)		37 (71%)	52 (70%)		
Extra-nodal			.70			.60	.50
>1	15 (12%)	18 (14%)		7 (13%)	13 (18%)		
≤1	111 (88%)	109 (86%)		45 (82%)	61 (82%)		
LDH (U/L)			.10			.40	.10
Abnormal	56 (44%)	69 (54%)		33 (63%)	40 (54%)		
Normal	70 (56%)	58 (46%)		19 (37%)	34 (46%)		
PS			.10			.90	.50
>1	1 (1%)	6 (5%)		2 (4%)	3 (4%)		
≤1	125 (99%)	121 (95%)		50 (96%)	71 (96%)		
Stage			.60			.20	<.01
I/II	56 (44%)	69 (54%)		33 (63%)	40 (54%)		
III/IV	70 (56%)	58 (46%)		19 (37%)	37 (46%)		
IPI score			.50			.50	.70
0	13 (10%)	15 (12%)		4 (8%)	8 (11%)		
1	39 (31%)	31 (24%)		15 (29%)	16 (22%)		
2	44 (35%)	48 (38%)		19 (36%)	28 (28%)		
3	22 (18%)	29 (23%)		11 (21%)	21 (28%)		
4	8 (6%)	4 (3%)		3 (6%)	1 (1%)		
IPI index			.40			.70	.40
>2	74 (59%)	81 (64%)		33 (63%)	50 (68%)		
≤2	52 (41%)	46 (36%)		19 (37%)	24 (32%)		
At relapse							
Age, median (range), yr	60 (19-79)	60 (23-76)	.90	56 (21-76)	54 (18-74)	.2	<.008
Extra-nodal			.30			<.009	.30
0	23 (18%)	15 (12%)		10 (19%)	13 (17%)		
1	88 (70%)	97 (76%)		40 (77%)	43 (58%)		
2	15 (12%)	15 (12%)		2 (4%)	17 (25%)		
3				0 (0%)	1 (2%)		
PS			.20			.90	.60
0	23 (18%)	15 (12%)		10 (19%)	13 (917%)		
1	88 (70%)	97 (76%)		40 (77%)	43 (58%)		
2	9 (7%)	14 (11%)		3 (5%)	5 (7%)		
LDH (U/L)	225 (75-1899)	230 (112-1065)	.10	240 (125-492)	238 (136-1102)	.9	.20
Stage			.90			.10	.20
I	17 (14%)	19 (15%)		13 (25%)	9 (12%)		
II	33 (26%)	30 (24%)		8 (15%)	21 (28%)		
III	20 (16%)	23 (18%)		15 (29%)	16 (22%)		
IV	56 (44%)	65 (43%)		16 (31%)	28 (38%)		
IPI risk factors							
Age, yr			.60			.60	<.004
>60	61 (48%)	57 (45%)		18 (35%)	21 (28%)		
≤60	65 (52%)	70 (55%)		34 (65%)	53 (72%)		
Extra-nodal			.90			<.002	.30
>1	15 (12%)	15 (12%)		2 (4%)	18 (24%)		

(*Continued on next page*)

Table 1
(*continued*)

Variable	Training Set n = 253			Validation Set n = 126			*P* Value*
	A-LMR ≥ 1 (n = 126)	A-LMR < 1 (n = 127)	*P* Value	A-LMR ≥ 1 (n = 52)	A-LMR < 1 (n = 74)	*P* Value	
≤1	111 (85%)	112 (88%)		50 (96%)	56 (76%)		
LDH (U/L)			<.01			.90	.08
Abnormal	47 (37%)	68 (54%)		29 (36%)	41 (55%)		
Normal	79 (63%)	59 (46%)		23 (44%)	35 (45%)		
PS			.40			.80	.40
>1	9 (7%)	14 (11%)		3 (6%)	5 (97%)		
≤1	117 (93%)	113 (89%)		49 (94%)	69 (93%)		
Stage			.90			.90	.80
I/II	50 (40%)	49 (39%)		31 (60%)	44 (59%)		
III/IV	76 (60%)	78 (61%)		21 (40%)	30 (41%)		
IPI score			.30			.08	.10
0	18 (14%)	16 (13%)		10 (19%)	16 (22%)		
1	43 (34%)	38 (30%)		12 (23%)	17 (23%)		
2	38 (30%)	33 (26%)		22 (42%)	22 (30%)		
3	18 (14%)	32 (25%)		8 (16%)	11 (15%)		
4	9 (8%)	7 (5%)		0 (0%)	6 (8%)		
5	0 (0%)	1 (1%)		0 (0%)	2 (2%)		
IPI index			.07			.20	.30
>2	27 (21%)	40 (32%)		8 (15%)	19 (26%)		
≤2	99 (79%)	87 (68%)		44 (85%)	55 (74%)		
Before transplantation							
Pretransplantation rituximab			.50			.90	.90
No	44 (35%)	49 (39%)		19 (37%)	26 (35%)		
Yes	82 (65%)	78 (61%)		33 (63%)	48 (65%)		
Pretransplantation treatments, n			.60			.20	.10
2	115 (91%)	117 (92%)		47 (90%)	50 (81%)		
3	10 (8%)	9 (7%)		5 (10%)	13 (18%)		
4	1 (1%)	1 (91%)		0 (0%)	1 (1%)		
Clinical status before transplantation			.06			.30	.30
CR	52 (41%)	37 (30%)		19 (37%)	23 (31%)		
PR	74 (59%)	90 (70%)		33 (63%)	51 (69%)		
At transplantation							
Age, median (range), yr	60 (24-78)	61 (20-77)	.50	57 (19-76)	54 (21-76)	.10	<.02
Plerixafor			.90			.70	.30
Yes	26 (20%)	26 (20%)		9 (17%)	11 (15%)		
No	100 (80%)	101 (80%)		43 (83%)	63 (85%)		
Conditioning regimens			<.02			.07	.60
CTX/TBI	2 (2%)	3 (92%)		0 (0%)	1 (1%)		
BEAC	9 (8%)	23 (18%)		10 (19%)	5 (7%)		
BEAM	115 (91%)	101 (80%)		42 (81%)	68 (92%)		
Infused CD34	4.5 (2.05-14.85)	4.2 (2.11-11.06)	.40	4.6 (2.12-11.37)	4.5 (2.03-11.52)	.90	.60
A-ALC, median (range)	.78 (.21-2.18)	.39 (.03-1.42)	<.0001	.80 (.32-2.61)	.39 (.04-2.01)	<.0001	.80
A-AMC, median (range)	.47 (.01-1.38)	.76 (.14-1.97)	<.0001	.49 (.11-1.90)	.75 (.24-2.41)	<.0001	.10
ALC-15, median (range)	.67 (.08-3.54)	.38 (.01-1.68)	<.0001	.77 (.09-2.64)	.39 (.05-2.14)	<.0001	.70
AMC-15, median (range)	.48 (.01-1.80)	.78 (.03-2.83)		.53 (.01-2.50)	.74 (.04-2.60)	<.0001	.10
LMR-15	1.37 (.25-31)	.57 (.02-9)	<.0001	.39 (.13-71)	.59 (.04-3.38)	<.0001	.20

U/L indicates upper limit; PS, performance status; PR, partial response; CTX, cyclophosphamide; TBI, total body irradiation; BEAC, BCNU, etoposide, ARA-C, and cyclophosphamide; BEAM, BCNU, etoposide, ARA-C, and melphalan.
Data presented are n (%) unless otherwise indicated.
* Difference between the training and validation set.

for the complete dataset used in the 10-fold procedure, by collecting the A-LMR, A-AMC, AMC-15, and LMR-15 obtained on each fold. For A-LMR, the cross-validation ROC (Figure 1B) showed an AUC of .73 (95% CI, .50 to .92); for A-AMC, an AUC of .63 (95% CI, .56 to .71); for AMC-15, an AUC of .66 (95% CI, .60 to .72); and for LMR-15, an AUC of .84 (95% CI, .80 to .88). The similar AUC from the empirical ROC and the cross-validation ROC support the use of A-LMR ≥1, A-AMC $\leq .5 \times 10^9$ cells/kg, AMC-15 ≤ 600 cells/μL, and LMR-15 ≥ 1 as the cut-off values as markers of the binary clinical outcome of death/survival.

Correlation between A-AMC and AMC-15 and between A-LMR and LMR-15 in the Training Set

We previously reported that ALC-15 recovery after APHSCT depended on the infused A-ALC. In this cohort of patients, a strong positive correlation between A-ALC and ALC-15 was observed in the training set ($R = .8$, $P < .0001$) and validated in the validation set ($R = .6$, $P < .0001$). The infusion of CD34 cells did not correlate with ALC-15 ($R = .1$, $P = .20$ in the training set and $R = .2$, $P = .10$ in the validation set). Therefore, we investigated if the A-AMC affects AMC-15 recovery as well as A-LMR directly affect LMR-15 after APHSCT. We identified a positive correlation between the infused A-AMC and AMC-15 ($R = .8$, $P < .0001$) in the training set and ($R = .8$, $P < .0001$) in the validation set. We also identified a positive correlation between A-LMR and LMR-15 ($R = .7$, $P < .0001$) in the training set (Figure 2A) and ($R = .6$, $P < .0001$) in the validation set (Figure 2B). Infused CD34 did not correlate with AMC-15 ($P = .10$ in the training set and $P = .60$ in the validation set) and LMR-15 ($P = .60$ in the training set and $P = .10$ in the validation set).

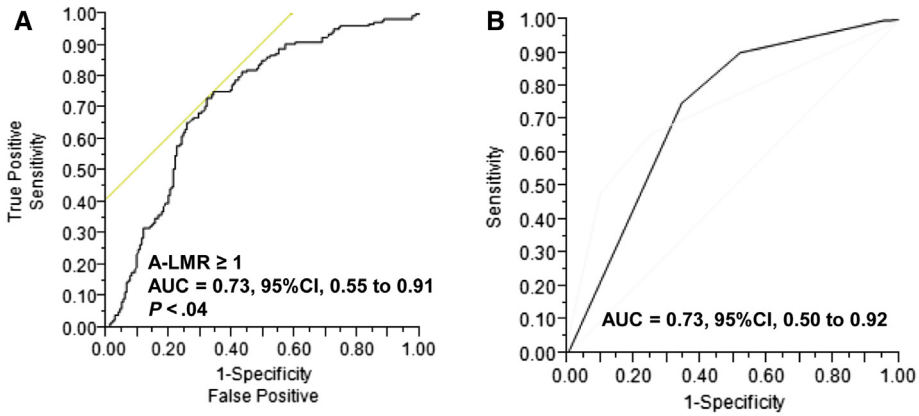

Figure 1. (A) Receiver operating characteristics curves (ROC) and area under the curve (AUC) for autograft lymphocyte to monocyte ratio (A-LMR) in the training set. (B) K-fold cross-validation ROC and AUC for A-LMR in the training set.

Predictors for OS, PFS, and LSS in the Training Set

Using the univariate Cox regression analysis, the following variables were predictors for OS, PFS, and LSS: LDH at diagnosis, LDH at relapse, stage at relapse, IPI at diagnosis, IPI at relapse, CR before transplantation, A-ALC, ALC-15, A-AMC, AMC-15, A-LMR, and LMR-15 (Table 2). Extranodal sites at relapse was a predictor for OS and PFS (Table 2). Multivariate analysis identified the following predictors for survival: A-ALC (OS, PFS, LSS), A-AMC (OS, PFS, LSS), A-LMR (OS, PFS, LSS), CR before transplantation (OS, PFS), LDH at relapse (PFS, LSS), and stage at relapse (PFS) (Table 3). To avoid the problem of colinearity due the strong positive correlation between A-ALC and ALC-15, between A-AMC and AMC-15, and between A-LMR and LMR-15, we substituted ALC-15 for A-ALC, AMC-15 for A-AMC, and LMR-15 for A-LMR and tested them against the other predictors (LDH at diagnosis, LDH at relapse, stage at relapse, IPI at diagnosis, IPI at relapse, and CR before transplantation) in the multivariate analysis. ALC-15 remained an independent predictor for OS (hazard ratio [HR], .49; 95% CI, .30 to .80; $P < .004$), for PFS (HR, .53; 95% CI, .36 to .79; $P < .002$), and for LSS (HR, .44; 95% CI, .24 to .79; $P < .005$); A-AMC for OS (HR, .40; 95% CI, .20 to .80; $P < .01$), for PFS (HR, .61; 95% CI, .37 to .97; $P < .04$), and for LSS (HR, .29; 95% CI, .13 to .66; $P < .003$); and LMR- 15 for OS (HR, .23; 95% CI, .11 to .44; $P < .0001$), for PFS (HR, .24; 95% CI, .15 to .40; $P < .0001$), and for LSS (HR, .12; 95% CI, .05 to .28; $P < .0001$).

The absolute monocyte count at diagnosis (AMC-D) has been also reported to be a predictor for survival in DLBCL. In the univariate analysis, we confirmed that AMC-D was a predictor for OS (HR, .55; 95% CI, .35 to .67; $P < .0001$), PFS (HR, .65; 95% CI, .41 to .91; $P < .01$), and LSS (HR, .41; 95% CI, .29 to .63; $P < .0002$). When we compared AMC-D against A-LMR in the multivariate analysis, A-LMR remained an independent predictor compared with AMC-D for OS (HR, .52; 95% CI, .31 to .87; $P < .01$), for PFS (HR, .61; 95% CI, .37 to .67; $P < .03$), and for LSS (HR, .34; 95% CI, .18 to .63; $P < .0004$).

Survival Outcomes Based on A-LMR

Using the cut-off value of 1.0 for the A-LMR obtained from the empiric ROC and subsequently validated by k-fold cross-validation in the training set, we tested A-LMR ≥ 1 for OS, PFS, and LSS. We observed that patients infused with an A-LMR ≥ 1 compared with patients infused with an A-LMR < 1 experienced superior OS (Figure 3A) and PFS (Figure 3B) in the training set (median OS was 167.2 months versus 17.6 months; 5-year OS, 73% [95% CI, 63% to 80%] versus 30% [95% CI, 22% to 38%], respectively; $P < .0001$; median PFS of 152.9 months versus 6.6 months; 5-year PFS of 67% [95% CI, 57% to 75%] versus 26% [95% CI, 18% to 34%], respectively; $P < .0001$). The superior OS and PFS observed in the group with an A-LMR ≥ 1 versus the group with an A-LMR < 1 was further validated in the validation set (median OS was 181.2 months versus 19.5 months; 5-year OS of 67% [95% CI,

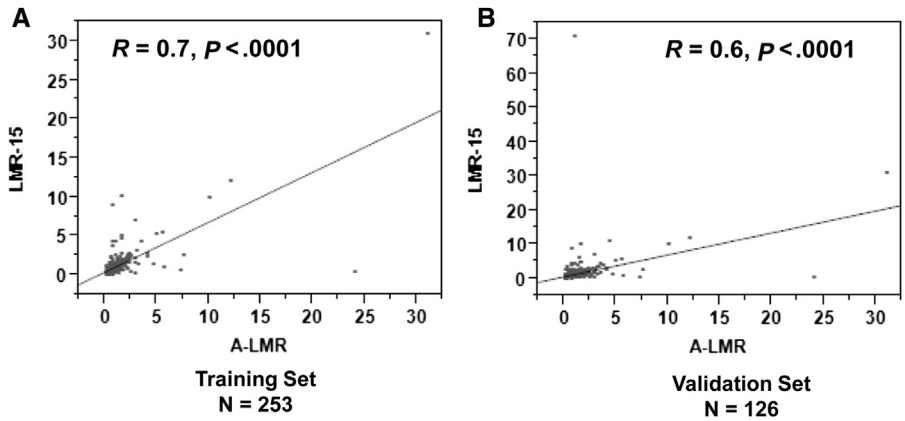

Figure 2. (A) Scatter plot of autograft lymphocyte to monocyte ratio (A-LMR) and lymphocyte to monocyte ratio at day 15 (LMR-15) after autologous peripheral hematopoietic stem cell transplantation in the training set. (B) Scatter plot of autograft lymphocyte to monocyte ratio (A-LMR) and lymphocyte to monocyte ratio at day 15 (LMR-15) after autologous peripheral hematopoietic stem cell transplantation in the validation set.

Table 2
Univariate Analysis for OS, PFS, and LSS in the Training Set

Variable	OS		PFS		LSS	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Age-D > 60	1.20 (.85-1.70)	.30	1.10 (.77-1.48)	.70	1.01 (.69-1.51)	.90
Age-R > 60	1.14 (.80-1.61)	.05	1.15 (.82-1.59)	.40	1.02 (.69-1.52)	.90
LDH-D (abnormal)	1.69 (1.91-2.43)	<.003	1.74 (1.25-2.44)	<.01	1.84 (1.24-2.76)	<.002
LDH-R (abnormal)	2.58 (1.87-3.72)	<.0001	2.61 (1.82-3.76)	<.0001	2.53 (1.71-3.82)	<.0001
Extra-nodal-D > 1	1.24 (.72-2.02)	.40	1.18 (.70-1.86)	.50	1.44 (.82-2.40)	.20
Extra-nodal-R > 1	1.79 (1.10-2.78)	<.02	1.58 (1.01-2.40)	<.05	1.69 (.92-2.78)	.06
PS-D > 1	1.33 (.41-3.15)	.60	2.03 (.86-1.03)	.10	1.24 (.31-3.31)	.70
PS-R > 1	2.91 (1.75-4.60)	<.0001	2.36 (1.43-3.71)	<.001	2.90 (1.63-4.83)	<.0006
Stage-D (III/IV)	1.20 (.82-1.80)	.30	1.24 (.87-1.80)	.20	1.30 (.85-2.08)	.20
Stage-R (III/IV)	2.18 (1.49-3.25)	<.0001	2.14 (1.50-3.10)	<.0001	2.14 (1.40-3.37)	<.0004
IPI-D > 2	1.65 (1.14-2.45)	<.008	1.75 (1.23-2.53)	<.002	1.74 (1.14-2.71)	<.009
IPI-R > 2	2.65 (1.85-3.79)	<.0001	2.44 (1.73-3.42)	<.0001	2.38 (1.59-3.55)	<.0001
CR before transplantation	.37 (.20-.64)	<.0002	.41 (.23-.67)	<.0002	.40 (.20-.73)	<.002
Rituximab before transplantation	.77 (.54-1.12)	.20	.85 (.60-1.20)	.40	.77 (.52-1.16)	.20
A-ALC $\geq .5 \times 10^9$/kg	.37 (.25-.52)	<.0001	.43 (.31-.60)	<.0001	.29 (.19-.43)	<.0001
A-AMC $\leq .5 \times 10^9$/kg	.58 (.39-.83)	<.003	.59 (.41-.83)	<.002	.51 (.32-.77)	<.001
A-LMR ≥ 1	.29 (.20-.43)	<.0001	.32 (.22-.45)	<.0001	.18 (.10-.28)	<.0001
ALC-15 $\geq$ 500 cells/μL	.30 (.20-.42)	<.0001	.36 (.25-.50)	<.0001	.25 (.16-.34)	<.0001
AMC-15 $\leq$ 600 cells/μL	.36 (.24-.52)	<.0001	.37 (.27-.51)	<.0001	.30 (.19-.45)	<.0001
LMR-15 ≥ 1	.13 (.08-.20)	<.0001	.14 (.10-.21)	<.0001	.07 (.03-.17)	<.0001

D indicates at diagnosis; R, at relapse.

52% to 79%] versus 35% [95% CI, 25% to 47%], respectively; $P <$ .0001; median PFS of 181.2 months versus 10.1 months; 5-year PFS of 65% [95% CI, 51% to 77%] versus 27% [95% CI, 18% to 39%], respectively; $P <$ .0001) (Figure 3D,E). Because of the long-term follow-up of 238.8 months in a group with a median age of 58 years, we analyzed the LSS to evaluate the direct impact of A-LMR on lymphoma survival, as patients in this cohort eventually died of other physical conditions not related to their APHSCT complications or lymphoma. In the training set, we observed superior LSS in patients with an A-LMR $\geq$1 in comparison with patients with an A-LMR <1 (median LSS was not-reached versus 18.6 months, 5-year LSS of 81% [95% CI, 72% to 88%] versus 33% [95% CI, 25% to 42%], respectively; $P <$ .0001) (Figure 3C). This observation was validated in the validation set (median LSS was not-reached versus 23.1 months, 5-year LSS of 71% [95% CI, 57% to 82%] versus 44% [95% CI, 32% to 56%], respectively, $P <$ .0001) (Figure 3F).

DISCUSSION

A-ALC has been reported to be a prognostic factor for survival after APHSCT [1-3], suggesting that host immunity affects clinical outcomes of patients treated with APHSCT. However, relapses have been reported in patients with infused A-ALC $\geq .5 \times 10^9$ cells/kg and long-lasting CR may occur in patients with infused low A-ALC $< .5 \times 10^9$ cells/kg [1]. Thus, we set out to investigate what other factors might counteract the superior clinical outcomes produced by A-ALC after APHSCT. Recent studies have shown the immunosuppressive and tumor growth effects of $CD14^+$ $HLA\text{-}DR^{low/neg}$ immunosuppressive monocytes. Because the A-AMC can be easily obtained from each apheresed unit, we combined A-ALC and A-AMC, as representative biomarkers of host immunity and immunosuppression, to study clinical outcomes after APHSCT in patients with DLBCL.

As in our previous publications, this study continues to show better clinical outcomes after APHSCT in DLBCL patients infused with an A-ALC $\geq .5 \times 10^9$ cells/kg. However, patients infused with an A-AMC $> .5 \times 10^9$ cells/kg also experienced inferior clinical outcomes. Combining both biomarkers, patients with an A-LMR $\geq$ 1 showed superior survival compared with those with an A-LMR <1. This finding was subsequently supported in a validation set. In multivariate analysis, it was revealed that A-LMR was an independent predictor for survival after APHSCT in the DLBCL.

We previously reported ALC-15 to be a prognostic factor for survival after APHSCT [21-25], which was confirmed by independent groups [26,27]. This initial observation led us to investigate the source of ALC-15 recovery. We subsequently

Table 3
Multivariate Analysis for OS, PFS, and LSS in the Training Set

Variable	OS		PFS		LSS	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
LDH-D (abnormal)	1.44 (.86-2.45)	.20	1.25 (.85-1.85)	.30	1.35 (.75-2.45)	.30
LDH-R (abnormal)	1.43 (.78-2.59)	.20	1.49 (1.03-2.16)	<.04	2.00 (1.40-3.47)	<.02
Extra-nodal-R > 1	1.34 (.66-2.87)	.40	1.23 (.73-2.10)	.40		
PS-R > 1	1.59 (.95-2.69)	.08	1.93 (.69-2.02)	.50	1.47 (.81-2.71)	.20
Stage-R (III/IV)	1.54 (.90-2.69)	.10	1.51 (1.08-2.30)	<.05	1.57 (.85-2.99)	.10
IPI-D > 2	1.03 (.59-1.80)	.90	1.03 (.67-1.59)	.90	1.09 (.58-2.03)	.80
IPI-R > 2	1.56 (.56-2.37)	.70	1.26 (.75-2.12)	.40	1.37 (.64-2.98)	.40
CR before transplantation	.50 (.27-.88)	<.02	.61 (.39-.92)	<.02	.60 (.29-1.14)	.10
A-ALC $\geq .5 \times 10^9$/kg	.56 (.36-.96)	<.03	.65 (.44-.96)	<.03	.37 (.17-.80)	<.01
A-AMC $\leq .5 \times 10^9$/kg	.48 (.25-.95)	<.04	.59 (.38-.92)	<.02	.53 (.29-.97)	<.04
A-LMR ≥ 1	.53 (.33-.87)	<.01	.57 (.38-.85)	<.006	.39 (.19-.78)	<.008

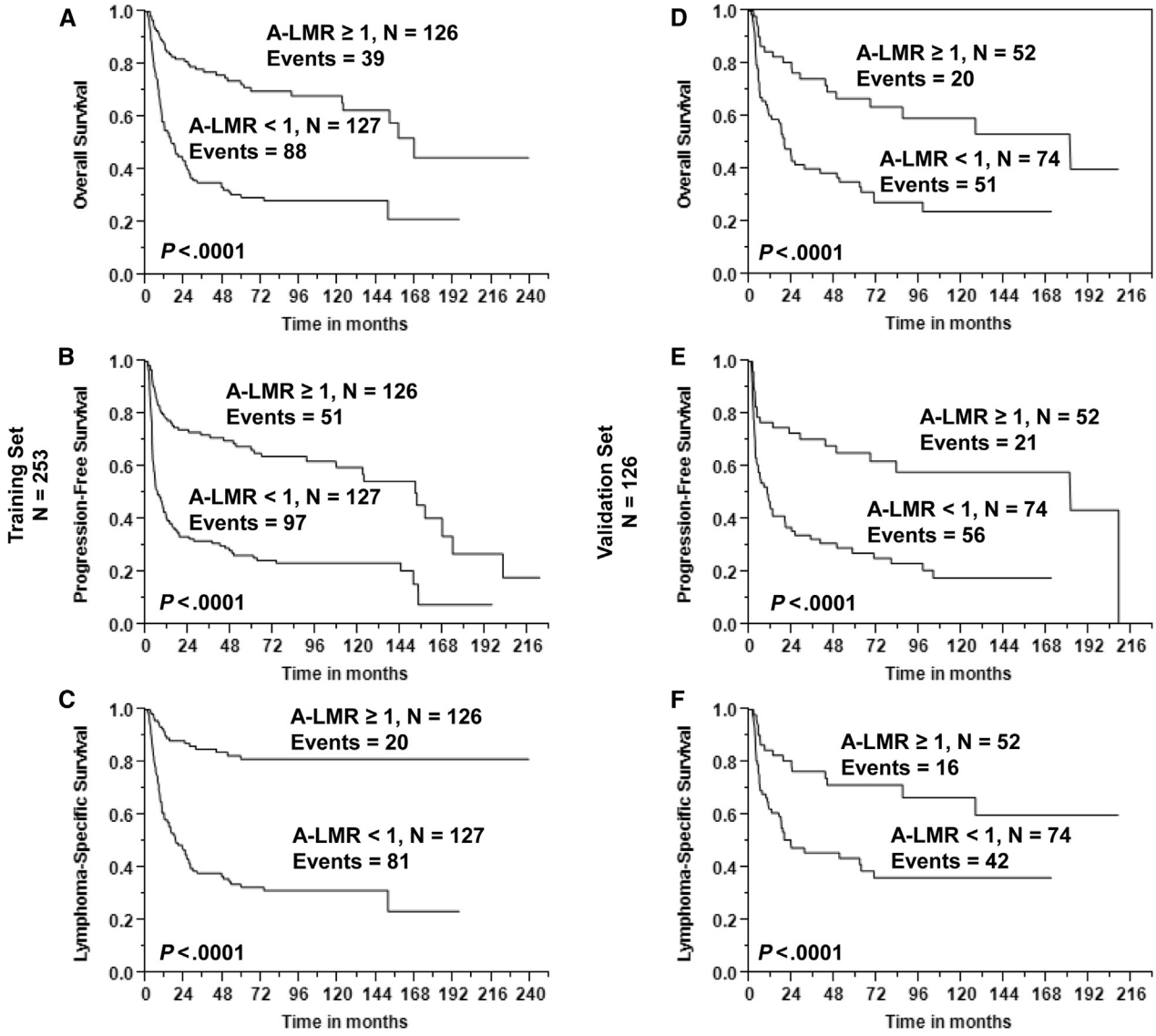

Figure 3. (A) Shows OS in the training set, (B) shows PFS in the training set, and (C) shows LSS in the training set. (D) Shows OS in the validation set, (E) shows PFS in the validation set, and (F) shows LSS in the validation set.

reported that ALC-15 recovery depends on the infused A-ALC [1,2], suggesting that immune effectors cells collected in the autograft directly affect immune recovery and survival after APHSCT. Thus, we investigated if the collected A-AMC and A-LMR correlated with AMC-15 and LMR-15. None of the patients in this study had any manipulation of their collected and infused autograft. We identified a strong positive correlation between the A-AMC and AMC-15, as well as A-LMR and LMR-15. No correlation was identified between CD34 and ALC-15, AMC-15, and LMR-15.

There are several possible mechanisms explaining why an A-LMR < 1 was associated with inferior survival after APHSCT by the infusion of high numbers of A-AMC. First, mobilized and collected monocytes have been shown to produce immunosuppressive cytokines (ie, IL-1β, IL-6, IL-10, TNF-α, transforming growth factor-β) dampening the host immune response [8,28,29]. Second, during cell-to-cell interaction, $CD14^+$ $HLA\text{-}DR^{low/neg}$ immunosuppressive monocytes disrupt the binding of specific peptide-major histocompatibility complex dimers to $CD8^+$ T cells through nitration of tyrosines by the production of peroxynitrite, rendering $CD8^+$ T cells unable to respond to specific peptides [30]. In allogeneic stem cell transplantation, downregulation of the expression of CD3ζ-chain has been reported by indoleamine 2, 3-dioxygenase produced by $CD14^+HLA\text{-}DR^{low/neg}$ cells. Blocking indoleamine 2, 3-dioxygenase restored the CD3ζ-chain expression, as well as increased production of interferon-ϒ, thus T cell activation [28]. No studies are available in APHSCT, but it is reasonable to hypothesize a similar process of T cell immunosuppression by the A-AMC could occur. Third, induction of regulatory T cells may be responsible. In a recent study in multiple myeloma patients mobilized with high-dose cyclophosphamide and G-CSF for APHSCT, researchers identified high levels in the autograft of $CD4^+$ $CD25^{high}$ regulatory T cells expressing high levels of Forkhead box P3 (FOXP3), cytotoxic-T-lymphocyte-associated protein 4, cytotoxic-T-lymphocyte-associated protein 4 (CTLA-4), and glucocorticoid-induced tumor-necrosis-factor-receptor-related protein (GITR), and displaying in vitro suppressive properties [31]. Fourth, upregulation of the death receptor Fas

may lead to T cell apoptosis [32]. Finally, decreased natural killer (NK) cells function is also possible. In vitro studies have shown that removal of granulocyte-macrophage colony-stimulating factor–mobilized or G-CSF–mobilized $CD14^+$ monocytes from the autograft increases NK cell function [33]. This is an important finding, as we previously published in a prospective study that NK cells are the main lymphocyte subset in the ALC-15 affecting survival after APHSCT [34]. Further studies are warranted to measure the $CD14^+$ HLA-$DR^{low/neg}$ immunosuppressive monocytes from the monocytic component collected in autografts to assess their impact on clinical outcomes.

To minimize the inherent biases of a retrospective study, such as selection bias and confounding factors, several steps were taken. In regard to selection bias, we included only patients with the unifying diagnosis of DLBCL to have a homogenous group. In addition, patients who received bone marrow harvest or combination of bone marrow harvest/peripheral blood stem cells were excluded. Only patients who received infused peripheral blood stem cells mobilized by G-CSF with or without plerixafor were included. Patients who participated in stem cell transplantation clinical trials were also excluded. Furthermore, patients were randomly divided into a training set and validation set to validate our findings. In regard to confounding factors, by the multivariate analysis, A-LMR remained an independent prognostic factor when compared with currently known prognostic factors for DLBCL patients undergoing APHSCT.

The strengths of the study include long-term follow-up of a uniform group of patients with DLBCL treated consecutively at a single institution. This study expands on the previous publications regarding A-ALC/ALC-15 by highlighting the importance of the interaction between host immunity and immunosuppression, using the simple biomarkers of A-ALC and A-AMC combined with the prognostic factor of A-LMR. Finally, the association between A-LMR and LMR-15, as well as A-AMC and AMC-15, provides a rationale to develop clinical translational interventions to engineer immunocompetent autograft with direct impact on immune recovery and survival after APHSCT. In regard to the collection of monocytes, G-SCF has been reported to mobilize and upregulate peripheral blood monocytes not only in patients undergoing APHSCT but also in normal donors for allogeneic stem cell transplantation. In allogeneic stem cell transplantation, the immunosuppressive effect of $CD14^+$ HLA-$DR^{low/neg}$ monocytes could be exploited to treat graft-versus-host disease. Even the use of plerixafor has been associated in the mobilization of high levels of monocytes in the peripheral blood [35]. Only 18% of patients received plerixafor in our cohort, rendering not enough events to perform meaningful statistical analysis to compare monocyte mobilization between patients who were mobilized only by G-CSF and those who were mobilized by G-CSF with plerixafor. Therefore, more targeted stem cell mobilization agents to mobilize stem cells, immune effector cells, and minimize collection of $CD14^+$ HLA-$DR^{low/neg}$ immunosuppressive monocytes might be a new avenue for a more immunocompetent autograft. Another option to reduce the infusion of $CD14^+$ immunosuppressive monocytes is to perform an autograft monocytic depletion, using a $CD14^+$ column to remove $CD14^+$ HLA-$DR^{low/neg}$ immunosuppressive monocytes from the autograft product.

In conclusion, if reproducible, the A-LMR has the potential to be an easily obtainable and universally applied biomarker to individually engineer immunocompetent autograft to improve clinical outcomes after APHSCT in DLBCL.

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