Human Pathology (2019) **86**, 21–31

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Human PATHOLOGY

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Original contribution

p53 expression in large B-cell lymphomas with *MYC* extra copies and CD99 expression in large B-cell lymphomas in relation to *MYC* status☆,☆☆

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Received 25 September 2018; revised 9 November 2018; accepted 15 November 2018

Keywords:
MYC;
p53;
CD99;
Large B-cell lymphoma;
MYC

Summary p53 expression and *MYC* extra copies (*MYC*-EC) have been reported to serve as independent adverse prognostic markers in patients with diffuse large B-cell lymphoma (DLBCL). However, the impact of p53 expression in *MYC*-EC lymphomas has not been delineated. Conversely, CD99 expression has been shown to have a positive impact on survival in patients with germinal center–type DLBCL, yet nothing is reported about the impact of CD99 expression and *MYC* status. This is the first study to evaluate p53 expression in *MYC*-EC lymphomas and CD99 expression in relation to *MYC* status. We identified 122 patients diagnosed as having large B-cell lymphoma (44, *MYC*-negative; 29, *MYC*-EC; 23, *MYC* rearrangement; 22, *MYC* and *BCL2* rearrangements; 4, *MYC*, *BCL2*, and *BCL6* rearrangements). p53 expression significantly correlated with DLBCL with abnormal MYC status (*MYC*-EC, *MYC* rearrangement, and MYC overexpression), but adverse p53 prognostic effect was only seen with *MYC*-rearranged lymphoma. CD99 expression was significantly associated with *MYC*-negative DLBCL and had better prognostic impact on lymphoma-specific survival (LSS), but not on relapse-free survival and overall survival. Overall, patients with *MYC*-EC lymphoma had significantly worse relapse-free survival and LSS than did patients with *MYC*-negative lymphoma, yet better overall survival and LSS than did the patients with *MYC*-rearranged lymphoma. Thus, patients with *MYC*-EC lymphomas had prognostic features that were intermediate between *MYC*-negative and *MYC*-rearranged lymphomas. Lastly, high-intensity chemotherapy (either dose-adjusted rituximab and etoposide-prednisone-vincristine-cyclophosphamide-doxorubicin or rituximab and hyperfractionated cyclophosphamide-vincristine-doxorubicin-dexamethasone treatment) did not improve survival in patients with *MYC*-EC and *MYC*-rearranged lymphoma when compared with rituximab-cyclophosphamide-hydroxydaunomycin-vincristine-prednisone therapy.

1. Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common (30%-40%) histologic subtype and perhaps the most heterogeneous type of non-Hodgkin lymphoma, and presents with a variety of clinical, morphologic, and molecular features. Currently, risk stratification assessment is based on the

☆ Competing interests: The authors declare no conflict of interest.

☆☆ Funding/Support: This project was supported in part by a Clinical Faculty Research Grant from the Department of Pathology & Laboratory Medicine, University of Kansas Medical Center to W. C.

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https://doi.org/10.1016/j.humpath.2018.11.015
0046-8177/

following: (1) International Prognostic Index (IPI), which uses information on patient age, performance status, serum lactate dehydrogenase (LDH) level, disease stage, and degree of extranodal involvement; (2) gene expression profiling, which divides DLBCL into 2 prognostically important subgroups of germinal center B-cell (GCB) and activated B-cell gene expression; and (3) identification of prognostic biomarkers that may contribute to better stratification of DLBCL, from which the most commonly analyzed are *MYC*, *BCL2*, and *BCL6* translocations.

In particular, aberrations of *MYC* are associated with an unfavorable prognosis and include gene translocation, *MYC* extra copies (*MYC*-ECs), or MYC protein overexpression [1-4]. Lymphomas with concurrent *MYC* and *BCL2* or *BCL6* rearrangements ("double-hit") and lymphomas with *MYC, BCL2* and *BCL6* rearrangements ("triple-hit") were shown to have highly aggressive clinical behavior with extremely poor outcome and resistance to chemotherapy [1,5-7]. The presence of *MYC*-EC is shown to be an independent poor prognostic factor, demonstrated by worse overall survival (OS) and progression-free survival (PFS) in the patients with DLBCL [8,9].

TP53 is a crucial tumor suppressor that limits oncogenesis by inducing cell-cycle arrest, DNA repair, and cell death. p53-dependent apoptosis serves as a rate-limiting step in *MYC* lymphomagenesis [10,11]. Inactivation of p53 suppressor pathway through mutation is associated with accelerated lymphomagenesis and drug resistance, resulting in aggressive lymphomas with poor prognosis [12,13]. *TP53* alteration and p53 expression have been shown to be independent markers of poor prognosis in patients with DLBCL [14,15]. Furthermore, p53 expression in association with *MYC* rearrangement (*MYC*-R) or MYC expression has demonstrated an addictive negative impact [14-16]. However, prognostic impact of p53 in lymphoma with *MYC*-EC has not been previously explored.

CD99 is a 32-kDa transmembrane protein with high-level surface expression on pediatric leukemias, Ewing sarcoma, anaplastic large cell lymphoma, and DLBCL. In contrast to the dismal outcome in patients with MYC and p53 overexpressed DLBCL, the role of CD99 in DLBCL is rather controversial. CD99-overexpressed DLBCLs have been reported to be associated with better event-free survival and OS within germinal center subgroup; however, others have reported CD99 expression to have an adverse impact on PFS in DLBCL patients [17,18].

Importantly, about one-third of DLBCLs, in particular DLBCL with high-grade features, experience more common treatment failure. Consequently, intensification of therapy as a means to improve outcomes in these patients is attempted by using dose-adjusted rituximab, etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin (R-EPOCH) or rituximab and hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone (R-HCVAD) and/or consolidation with high-dose therapy and transplant [19,20]. However, the results have been mixed, the benefits have been debated, and development of novel drugs is still needed to improve outcomes.

The purpose of this study is to define prognostic impact of p53 expression and CD99 expression in patients with large B-cell lymphoma harboring *MYC*-EC, or *MYC*-R alone or together with *BCL2*− and/or *BCL6*− rearrangement(s). In addition, we evaluate prognostic outcomes of patients depending on *MYC* status by comparing standard treatment with rituximab, cyclophosphamide, hydroxydaunomycin, and vincristine-prednisone (R-CHOP) and intensive chemotherapy (R-EPOCH and R-HCVAD). To the best of our knowledge, this is the first study to evaluate p53 expression in lymphomas with *MYC*-EC and CD99 expression in relation to *MYC* status.

2. Materials and methods

2.1. Case selection

We retrospectively performed keyword search in our electronic record system of Kansas University Medical Center and identified 122 patients as having with large B-cell lymphoma from 2001 to 2016. The initial diagnosis of lymphoma in 2 patients was made in 1990 and 1995, respectively, and later, both patients presented with recurrence. Our study included 41 cases of DLBCLs with *MYC* normal (*MYC*-N); 28 cases of DLBCLs with *MYC*-EC (23 with 3-4 copies and 5 with ≥5 copies of MYC); 22 cases of DLBCLs with *MYC*-R; 5 cases of high-grade B-cell lymphoma, not otherwise specified (NOS; 3 for *MYC*-N; 1, *MYC*-R; and 1, *MYC*-EC); 22 cases of high-grade B-cell lymphoma with *MYC* and *BCL2* or *BCL6* rearrangements (double-hit lymphoma; DH); and 4 cases of high-grade B-cell lymphoma with *MYC/BCL2/BCL6* rearrangements (triple-hit lymphoma; TH). All diagnoses were made according to 2016 World Health Organization classification [21]. Clinical, morphologic, immunophenotypic, and cytogenetic data were reviewed. This retrospective study was approved by the institutional review board.

2.2. Immunostaining

Immunohistochemical analysis was performed using formalin-fixed, paraffin-embedded tissue sections. We assessed expression of MYC, CD99, and p53 using the following antibodies: c-Myc (EP121) rabbit monoclonal antibody (Biocare Medical, Epitomics), CD99 (EP8) rabbit monoclonal antibody (Cell Marque, Epitomics), and p53 (DO-7) mouse monoclonal antibody (Dako). Primary antibodies were used at the following concentrations: c-Myc (1:50), CD99 (1:200), and p53 (ready to use, undiluted). Procedures were performed at room temperature using the Biocare IntelliPath autostainer. Epitope retrieval was performed using the Biocare Decloaking Chamber. Slides were counterstained with hematoxylin and permanently mounted.

We used the following cutoffs for reporting positive protein expression: ≥40% for MYC, ≥25% for CD99, ≥50% for p53, and ≥50% for BCL-2, respectively [21,22]. The panel of

antibodies also included CD3 (2GV6); CD5 (SP19), both rabbit monoclonal antibody (Ventana); CD10 (56C6) mouse monoclonal (Cell Marque); CD20 (L26) mouse monoclonal (Ventana); BCL2 (124) mouse monoclonal (Dako); BCL6 (GI191E/A8) mouse monoclonal (Ventana); MUM1 (MUM1p) mouse monoclonal (Dako); and Ki-67 (MIB-1) mouse monoclonal (Dako). The GCB or the non-GCB of DLBCL was assigned using 30% cutoffs for BCL6, CD10, and MUM1 as defined using the algorithm by Hans et al [23].

2.3. Conventional cytogenetic studies and fluorescence in situ hybridization

Cytogenetic testing, either by conventional karyotype or by fluorescence in situ hybridization (FISH), was performed on diagnostic biopsy specimens at initial workup or retrospective study. The conventional karyotypes were reported according to the 2016 International System for Human Cytogenetic Nomenclature.

FISH was performed on unstained slides with an *MYC* and a *BCL6* dual-color break-apart probe set and an *IGH/BCL2* dual-color-fusion probe (Abbott Molecular, Des Plaines, IL) following the manufacturer's protocol. For bone marrow aspirate specimens, FISH was performed by using a freshly dropped slide from a harvested bone marrow aspirate specimen. For formalin-fixed, paraffin-embedded tissue samples, FISH was performed on 4-mm-thick tissue sections and fixed onto slides as per the manufacturer's protocols.

A total of 100 interphase nuclei per case were scored by 2 independent observers using a fluorescence microscope (Olympus BX 61; Olympus America, Center Valley, PA) at ×60 magnification with oil immersion. *MYC-EC* was defined as cells with ≥3 to 4 signals of *MYC* in ≥10% of nuclei. The case was defined as positive for *MYC, BCL2*, or *BCL6* rearrangement if ≥4.6%, ≥3.0%, or ≥3.0% of nuclei showed positive signals, respectively. The cutoff values were established in normal patient tissue in our laboratory.

2.4. Statistical analysis

OS was defined as the date of diagnosis to the date of death from any cause. Relapse-free survival (RFS) was defined as the time from the date of diagnosis to the date of first recurrence or death. Lymphoma-specific survival (LSS) was calculated from the date of diagnosis to the date of death as a result of lymphoma (patients who died of a cause other than lymphomas were censored). Patients who were lost to follow-up were censored on their last follow-up date. Patient survival was analyzed using the Kaplan-Meier method and compared using the log-rank test, and multivariate analysis was performed using Cox regression test (IBM SPSS, Chicago, IL). χ^2 Test was performed using the online GraphPad calculator (GraphPad Software, La Jolla, CA). A P value of $<.05$ was considered statistically significant.

3. Results

3.1. Overall clinical characteristics

We retrospectively analyzed a total of 122 patients, of which 91 patients were diagnosed as having DLBCL, NOS, 5 patients were diagnosed as having high-grade B-cell lymphoma , NOS (3 with *MYC*-N, 1 with *MYC*-EC and 1 with *MYC*-R), 22 patients were diagnosed as having high-grade B-cell lymphoma with *MYC* and *BCL2* or *BCL6* rearrangements, and 4 patients were diagnosed as having high-grade B-cell lymphoma with *MYC*, *BCL2*, and *BCL6* rearrangements. Clinical characteristics—including age, sex, clinical stage, serum LDH level, number of extranodal sites involvement, performance status of Eastern Cooperative Oncology Group (performance status), IPI score, bone marrow status, central nervous system (CNS) involvement, treatment regimens, genetic status of *BCL2* and *BCL6*, and immunohistochemical characterization of CD99, MYC, p53, and BCL-2 expression—are summarized in Table 1. The patients included 70 men and 52 women with a mean age of 61 years (range, 26-91 years). There was no significant difference between these 3 groups regarding age, sex, stage, and CNS involvement. Supplementary Fig. 1 shows expression of MYC, p53, and CD99 and *MYC* status in *MYC*-N, *MYC*-R, and *MYC*-EC lymphomas.

3.2. Clinicopathological features of *MYC*-EC versus *MYC*-R versus *MYC*-N B-cell lymphoma

The incidence of *MYC*-EC lymphoma in our study was 23.7% (29/122). Patients with *MYC*-EC lymphoma were significantly different from *MYC*-N in following clinicopathological features: elevated LDH, higher frequency of MYC and p53 expression, MYC and BCL2 double-expression, decreased frequency of CD99 expression, and less complete response (CR); yet, there was no significant difference in chemotherapy regimens or disease relapse. When compared with the *MYC*-R B-cell lymphoma, patients with *MYC*-EC B-cell lymphoma showed less bone marrow involvement, lower frequency of MYC expression, and less association with GCB subtype of cell of origin (COO) and received less intensive induction chemotherapy (R-EPOCH or R-HCVAD); yet, there was no significant difference in CR or disease relapse.

Only limited information on genetic status of *BCL2* and *BCL6* in MYC-EC (n = 6) and MYC-N (n = 13) groups was available; there was no significant difference in incidence when compared with each other or compared with the MYC-R group.

The incidence of *MYC*-R lymphoma in our study was 40% (49/122), which included 22 cases of DLBCL, 1 case of high-grade B-cell lymphoma, NOS, 22 cases of high-grade B-cell lymphoma with *MYC/BCL2* or *MYC/BCL6* rearrangements, and 4 cases of high-grade B-cell lymphoma with *MYC/BCL2/BCL6* rearrangements. Similar to previous findings, patients with *MYC*-R lymphoma showed significantly elevated

Table 1 Clinicopathological features of *MYC*-N, *MYC*-EC, and *MYC*-R in DLBCL

	N (n = 44)	EC (n = 29)	R (n = 49)	EC *vs* N	EC *vs* R	N *vs* R
Age (y)						
Median (range)	58.5 (26-91)	61 (38-83)	62 (42-88)			
<60	23	12	20	NS	NS	NS
>60	21	17	29			
Sex						
Male	25	15	30	NS	NS	NS
Female	19	14	19			
Stage						
I-II	16	7	10			
III-IV	25	21	35	NS	NS	NS
No data	3	1	4			
LDH						
Normal	21	6	8			
Elevated	20	19	35	.0291	NS	.0017
No data	3	4	6			
No. of extranodal						
Sites	27	18	21			
0-1	12	10	24	NS	NS	.0371
≥2	5	1	4			
No data	0	0	0			
PS						
0-1	34	20	28			
≥2	5	6	15	NS	NS	.0236
No data	5	3	6			
IPI score						
0-2	25	11	15			
3-5	14	14	28	NS	NS	.0082
No data	5	4	6			
BM						
No	26	21	21	NS	.011	NS
Yes	18	8	28			
CNS						
No	42	25	44	NS	NS	NS
Yes	2	4	5			
COO						
GCB	24	16	44	NS	.0004	.0001
Non-GCB	20	13	5			
Therapy						
R-CHOP	36	21	16	NS	.0004	<.00001
R-EPOCH	0	1	14	–	–	–
R-HCVAD	3	2	8	–	–	–
Other	5	3	8	–	–	–
No data	0	2	3			
CR						
Yes	35	16	23			
No	6	9	22	.0451	NS	.0007
No data	3	4	4			
Relapsed						
Yes	14	12	28	NS	NS	.0143
No	30	17	21			
CD99						
CD99+	24	7	7	.0150	NS	.00007
CD99−	18	19	37			
No data	2	3	5			
P53						
P53+	2	8	21	.005	NS	.0001

Table 1 (*continued*)

	N (n = 44)	EC (n = 29)	R (n = 49)	EC *vs* N	EC *vs* R	N *vs* R
P53−	40	18	24			
No data	2	3	4			
MYC						
MYC+	13	21	48	.0003	.0015	<.00001
MYC−	29	7	1			
No data	2	1	0			
Bcl2						
Bcl2+	27	22	36	NS	NS	NS
Bcl2−	9	3	12			
No data	7	4	1			
DE						
DE(MYC+/Bcl2+)	7	16	35	.0004	NS	.0009
Non-DE	29	8	13			
No data	8	5	1			
BCL2-R						
BCL2-R+	3	1	19	NS	NS	NS
BCL2-R−	10	5	30			
No data	31	38	0			
BCL6-R						
BCL6-R+	1	0	7	NS	NS	NS
BCL6-R−	12	6	42			
No data	31	38	0			

Abbreviations: BM, bone marrow involvement; EC, *MYC* extra copy; N, *MYC* normal; NS, nonsignificant; PS, performance status; R, *MYC* rearranged.

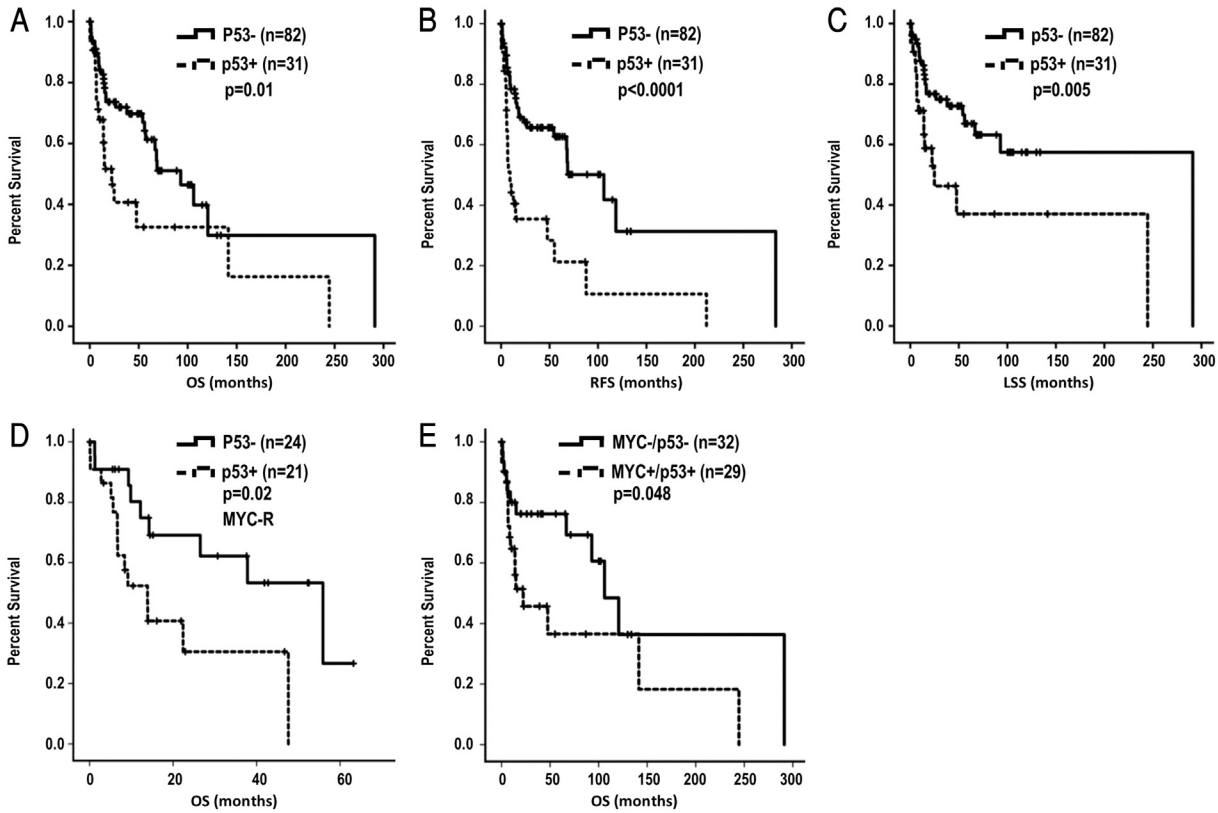

Fig. 1 Survival comparison between p53-positive and p53-negative B-cell lymphomas. A, OS. B, RFS. C, LSS. D, OS in *MYC*-R lymphomas. E, OS in lymphomas with different MYC expression.

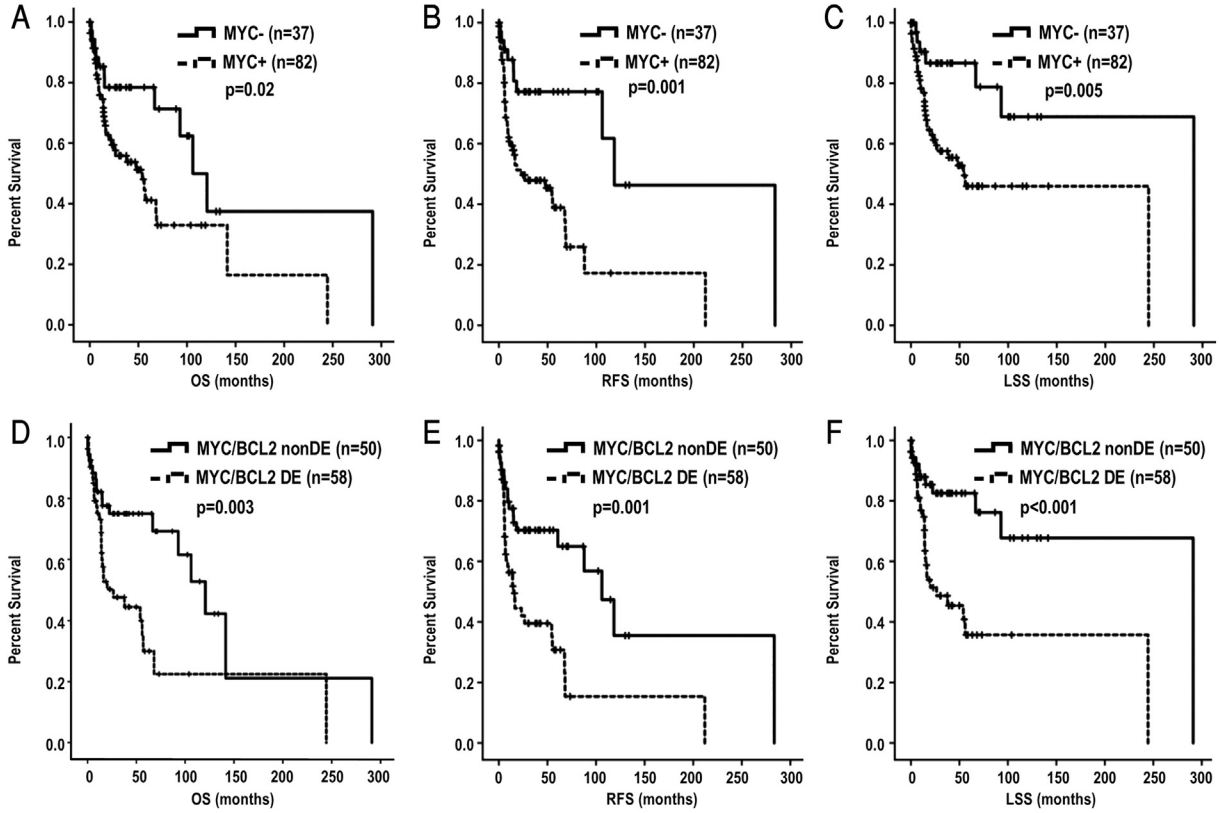

Fig. 2 Survival comparison between MYC-positive and MYC-negative B-cell lymphomas. A, OS. B, RFS. C, LSS. D, OS in lymphomas with different BCL2 expression. E, RFS in lymphomas with different BCL2 expression. F, LSS in lymphomas with different BCL2 expression.

LDH level, increased extranodal site involvement, higher IPI score, poorer performance status, higher frequency of MYC and p53 expression as well as MYC and BCL2 double expression, decreased frequency of CD99 expression, and significantly higher association with GCB subtype. In addition, patients with *MYC*-R lymphoma received more commonly intensive induction therapy (R-EPOCH or R-HCVAD) but had significantly lower CR to treatment and relapsed more commonly compared with patients with *MYC*-N lymphoma.

3.3. Evaluation of expression of p53, MYC, and CD99 and their prognostic impacts

p53 expression was identified in 27.8% (32/115) of the patients in our study. Notably, the frequency of p53 expression was significantly higher in *MYC*-EC, *MYC*-R lymphomas (Table 1), and MYC-expressing lymphomas (Supplementary Table 1). When evaluating for prognostic effect of p53 expression, patients with p53-expressed B-cell lymphoma had inferior OS, RFS, and LSS compared with patients with p53-negative B-cell lymphoma (Fig. 1A-C). However, the adverse impact on OS by p53 expression was only demonstrated in the *MYC*-R group (Fig. 1D), but not in the *MYC*-EC and *MYC*-N groups (data not shown). Since only 2 and 8 cases showed p53 expression in the latter 2 groups, the negative findings in these 2 groups might not be statistically significant. In addition, patients with lymphomas with double expression of MYC and p53 showed worse OS compared with patients with lymphomas without MYC and p53 expression (Fig. 1E).

MYC expression was identified in 67.6% (73/108) of cases in our study. Consistent with previous findings, patients with MYC-expressing lymphomas (Fig. 2A-C) and patients with MYC/BCL2 double-expressing lymphomas (DE) (Fig. 2D-F) had significantly poorer OS, RFS, and LSS when compared with MYC-negative lymphoma, respectively. Patients with DE lymphomas showed similarly poor OS when compared with patients with *MYC* and *BCL-2* and or *BCL-6* DH/TH (Supplementary Fig. 2A).

Lymphoma with CD99 expression was identified in 33.92% (38/112) of the cases in our study. The frequency of CD99 expression was highest in patients with *MYC*-N lymphoma (58.54%) and lowest in patients with *MYC*-R (15.9%). CD99 expression was identified in 25.9% of patients with *MYC*-EC lymphomas. There was no significant difference in CD99 expression between patients with *MYC*-EC and *MYC*-R lymphomas. Furthermore, analyses of CD99-expressing lymphoma based on COO classification failed to show any significant association between CD99 expression

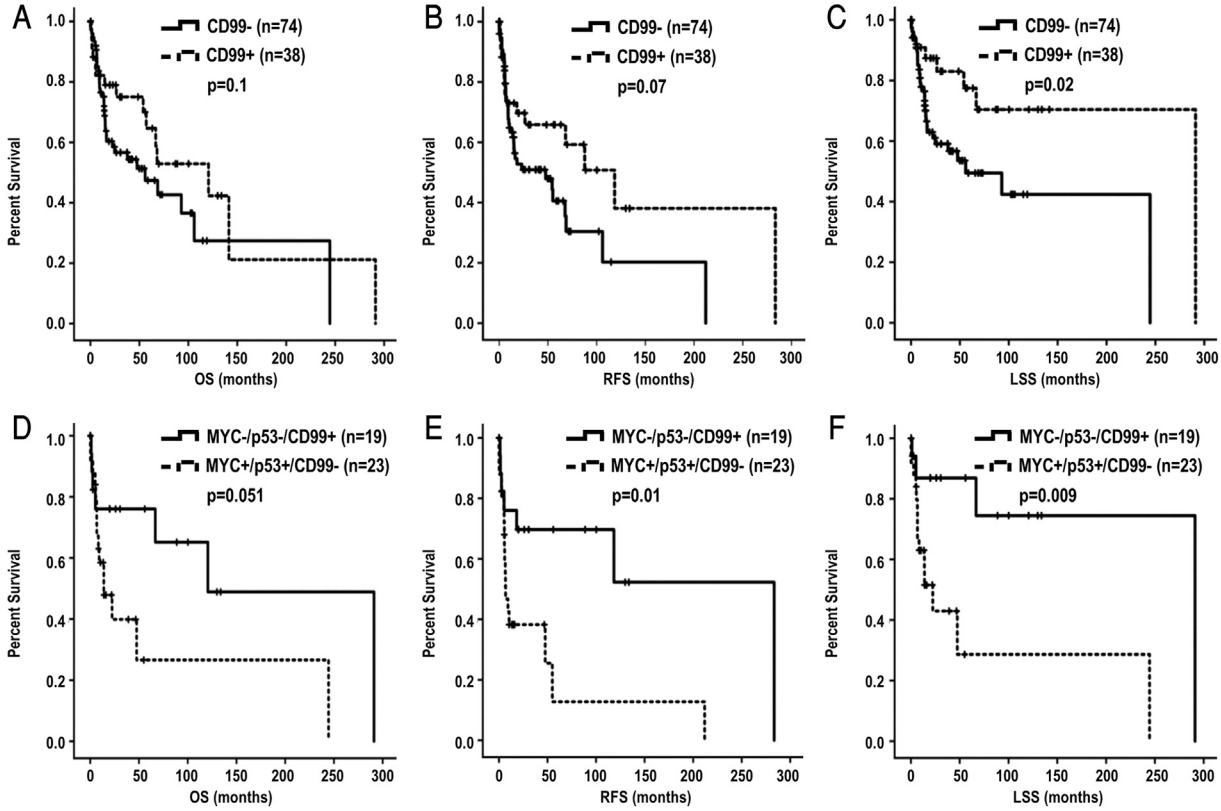

Fig. 3 Prognostic impact of CD99 expression on survivals. A, OS for the entire patient cohort. B, RFS for the entire patient cohort. C, LSS for the entire patient cohort. D, OS comparison in patients with different MYC and p53 expression. E, RFS comparison in patients with different MYC and p53 expression. F, LSS in patients with different MYC and p53 expression.

and COO classification (P = .2162). Lastly, the expression of CD99 had a positive impact on LSS, but not on OS and RFS in our study (Fig. 3A-C).

We also analyzed if CD99 expression could improve survival in MYC and p53 double-negative lymphomas when compared with MYC and p53-DE lymphomas. Interestingly, CD99 expression significantly improved survival benefits in MYC and p53 double-negative lymphomas (Fig. 3D-F). These findings further indicated that CD99 expression could serve as a favorable prognostic factor.

3.4. The status of *MYC* and its prognostic impact

Patients with *MYC*-R B-cell lymphoma had inferior OS, RFS, and LSS than did patients with *MYC*-N cell lymphoma (Fig. 4A-C). Patients with *MYC*-EC B-cell lymphoma had inferior RFS and LSS than did patients with *MYC*-N B-cell lymphoma but better OS and LSS than did patients with *MYC*-R B-cell lymphomas. The negative impact of *MYC*-EC was unrelated to the presence of either 3 to 4 copies or >5 copies (P > .05; data not shown).

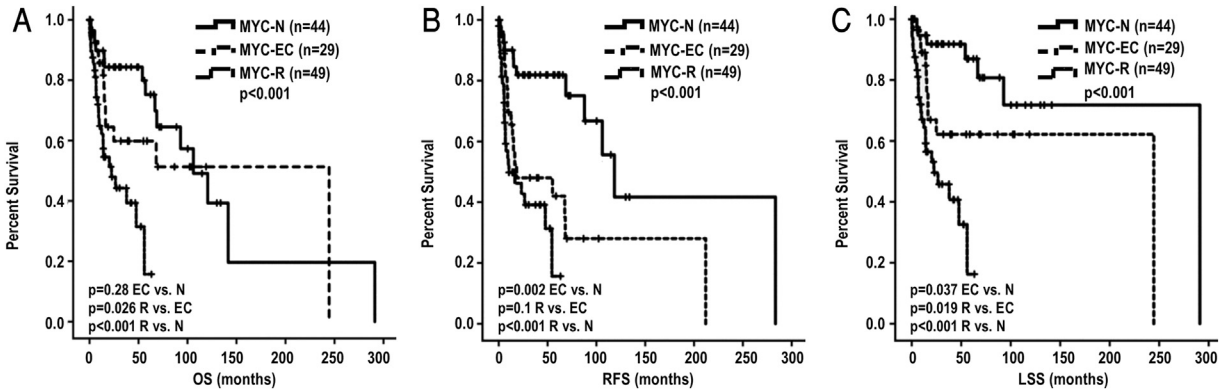

Fig. 4 Survival comparison in B-cell lymphomas with different *MYC* genetic background. A, OS. B, RFS. C, LSS.

Table 2 Univariate and multivariate analyses of clinicopathological parameters on OS, RFS, and LSS

Variables	Univariate, *P*	Multivariate analysis		
		HR	95% CI	*P*
Entire patient cohort				
OS				
MYC-EC	NS	1.66	0.61-2.92	NS
MYC-R	<.001	3.09	1.48-6.45	.003
IPI scores	<.001	3.72	2.00-6.94	<.001
RFS				
MYC-EC	NS	3.04	1.39-6.65	.005
MYC-R	.002	4.01	1.85-8.67	<.001
IPI scores	<.001	3.72	2.05-6.73	<.001
LSS				
MYC-EC	NS	2.45	0.88-6.82	.09
MYC-R	<.001	5.90	2.28-15.26	<.001
IPI scores	<.001	3.36	1.69-6.69	.001
MYC-N and *MYC*-R cohort				
OS				
P53 expression	<.001	2.53	1.23-5.17	.01
MYC-R	<.001	3.07	1.28-7.40	.01
IPI scores	<.001	4.34	2.08-9.08	<.001
RFS				
P53 expression	<.001	3.87	1.85-8.10	<.001
MYC-R	<.001	3.00	1.24-7.23	.02
IPI scores	<.001	3.73	1.80-7.73	<.001
LSS				
P53 expression	<.001	2.41	1.09-5.39	.03
MYC-R	<.001	6.14	2.04-18.6	.001
IPI scores	<.001	3.66	1.62-8.25	.002

Abbreviation: NS, no significance.

Patients with DH/TH lymphomas did not show worse OS than did the rest of the patients (Supplementary Fig. 2B). Li et al [16] demonstrated that patients with single-hit DLBCL did not show difference in OS when compared with patients with double-hit lymphomas. Our finding of lack of survival difference could be explained by nearly one-quarter of the latter group composed of single-hit lymphoma.

3.5. Univariate and multivariate analyses

Univariate and multivariate analyses performed in all patients revealed that higher IPI scores and *MYC*-R were significantly associated with worse OS, RFS, and LSS (Table 2). Notably, although patients with *MYC*-EC lymphoma failed to demonstrate inferior survivals by univariate analysis, these patients indeed showed significantly worse RFS ($P = .005$; Table 2) and a trend for worse LSS ($P = .09$) by multivariate analysis.

Univariate and multivariate analyses performed in patients with *MYC*-N or *MYC*-R lymphoma showed that higher IPI scores, *MYC*-R, and p53 expression were significantly associated with worse OS, RFS, and LSS (Table 2). Interestingly, the association of p53 expression with worse survivals demonstrated by multivariate analysis was only present in this particular group of patients. p53 enhanced the negative impact of *MYC*-R (Fig. 1E) on survival synergistically but exerted no addictively adverse effect on survivals in patients with *MYC*-EC lymphoma (Fig. 1F). These findings indicate that the interaction of MYC and p53 may be highly dependent on *MYC* genetic background. The findings also further support the notion that p53 expression and *MYC*-R can serve as independent prognostic markers.

3.6. CHOP therapy versus intensive therapy

We next evaluated the treatment response to different chemotherapeutic regiments. A total of 73 patients received treatment with R-CHOP and 29 patients received more aggressive treatment regimens, of which 14 patients received R-HCVAD and 15 patients received R-EPOCH. Patients treated with R-CHOP therapy showed survival benefit when compared with the group treated with intensive therapy (Fig. 5A). However, when we further divided the patient group into *MYC*-R and *MYC*-EC groups, there was no difference in OS in both the *MYC*-R and *MYC*-EC groups when treated with either therapy (Fig. 5B and C).

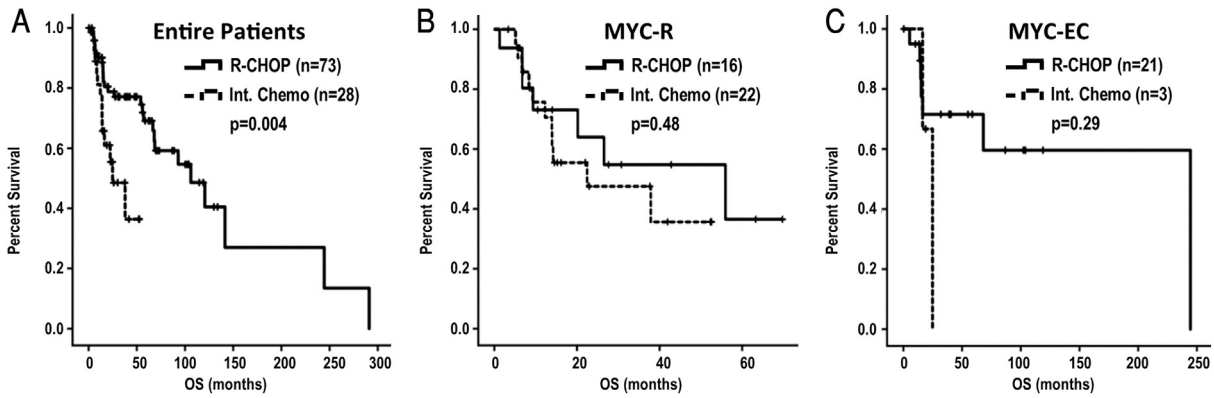

Fig. 5 OS comparison in B-cell lymphomas treated with different chemotherapy regimens. A, Entire patient cohort. B, Patients with *MYC*-R lymphomas. C, Patients with *MYC*-EC lymphomas.

4. Discussion

The role of increased *MYC* copy numbers in lymphoma is not well understood, and the data are somewhat conflicting due to limited studies available for review. Some studies have shown that *MYC*-EC is associated with inferior OS, whereas others have shown that patients with *MYC*-EC have poor OS and PFS only if concomitant del(8p) is present [9,14,24,25]. However, similar OS in patients without extra *MYC* copies is also reported [26,27]. In addition, some studies suggest that >5 copies are needed for poor prognosis, not 3 to 4 copies, yet another study has shown that patients with increased *MYC* copy number, regardless of copy number, have worse OS [14,28]. In our study, we do not observe difference in OS, RFS, or LSS between 3 to 4 copies and ≥5 copies of *MYC*. We demonstrate that patients with *MYC*-EC lymphomas have significantly worse RFS and LSS than do patients with *MYC*-N lymphoma but better OS and LSS than do patients with *MYC*-R lymphoma. Therefore, *MYC*-EC lymphoma represents a unique group of lymphoma with prognostic features that are intermediate between *MYC*-N and *MYC*-R lymphomas.

In our study, overexpression of MYC based on immunostaining occurred in 67.6% of patients with DLBCL, of which 58.3% can be explained by the presence of *MYC* alterations (18.3% with *MYC*-EC and 40% with *MYC*-R), leaving 10.8% of *MYC*-N lymphoma showing MYC expression. This discordance suggests that mechanisms other than gene alterations are responsible for protein overexpression in a proportion of DLBCL cases.

Immunohistochemical staining with >50% cells positive for p53 has been reported to be a good surrogate marker for *TP53* mutation and can be used to stratify patients with significantly different prognoses [22]. In our study, the frequency of p53 expression in DLBCL is 27% using a 50% cutoff of positive cells, which is similar to other studies in which 20% to 40% DLBCLs are reported to be positive for p53 expression [14,29,30]. We demonstrate that p53 expression is associated with a poorer OS, RFS, and LSS in DLBCL patients by univariate analysis. We also demonstrate that p53 expression is an independent prognostic marker in the group of patients with *MYC*-N or *MYC*-R DLBCL by multivariate analysis.

Furthermore, p53 expression is significantly associated with *MYC* aberration and MYC expression. p53 expression enhances the negative impact of *MYC* rearrangement on survivals synergistically. These findings are consistent with previous reports [14,31]. However, in our study, the negative synergistic effect is not demonstrated in patients with *MYC*-EC lymphoma. The findings might be due to a small sample size in the group of *MYC*-EC with p53 expression (n = 8). The interaction between MYC and p53 has been previously investigated. Qi et al [32] demonstrated a multiprotein complex comprising ARF-BP1, ARF, p53, MYC, and the multifunctional DNA-binding factor, CTCF, by coimmunoprecipitation studies. Here, MYC provides a constitutive proliferative signal but can also initiate ARF-dependent activation of p53 and apoptosis [10,11]. On the other hand, wild-type p53 can induce microRNA124, a negative regulator of MYC and BCL2, and therefore downregulates expression of MYC and BCL2 [33]. However, in the presence of *TP53* mutation, neither MYC-initiated p53 activation and apoptosis nor miRNA-dependent negative regulation of MYC and BCL2 can occur. When tumor harbors both *MYC* translocation and *TP53* mutation, only MYC-driven proliferation prevails [11,12].

Previous study has shown that both CD99-positive expression in GCB subtype and CD99-negative expression in non-GCB subtype result in superior 2-year event-free survival and 2-year OS, respectively, when compared with other groups [17]. However, Lee et al [18] have found significant association of CD99 positivity with non-GCB subtype and poor PFS. In our study, CD99 expression significantly associates with *MYC*-N DLBCL and fails to show any association with either GCB or non-GCB COO. CD99-overexpressed DLBCL has significantly better LSS and a trend of better RFS (P = .07). Furthermore, CD99 expression also improves survival benefit in MYC− and p53−double-negative B-cell lymphomas.

Various strategies have been implemented to improve the treatment response in DLBCL, particularly, in high-grade DLBCL, with mixed results. Some studies have shown that a subset of patients might benefit from intensive induction with or without transplant. Landsburg et al have reported an inferior 3-year RFS for patients treated with R-CHOP compared with those treated with intensive front-line therapy [27]. Howlett et al have similar findings where first-line treatment with R-EPOCH has significantly reduced the risk of disease progression compared with R-CHOP [19]. However, OS is not significantly different across treatment approaches for either DLBCL with *MYC*-R only or with *MYC* and *BCL2* rearrangements (single-hit and double-hit lymphoma) [19,27]. In our study, neither patients with *MYC*-EC nor patients with *MYC*-R lymphoma show significant difference in OS when comparing R-CHOP with intensive chemotherapy. Our findings are in agreement with large phase 3 multicenter study (CALGB 50303) that compares R-CHOP and DA-EPOCH-R regimens in DLBCL patients and finds no difference in event-free survival or OS [34]. While both regimens achieve similar efficacy in event-free survival and OS, RCHOP is associated with less toxicity and greater convenience as an outpatient treatment. Our study demonstrates survival benefit in RCHOP treated patients over intensive regimen treated patients when we analyze the entire patient population, however, this benefit is confounded by different genetic background since intensive regimen treated group is mainly composed of *MYC*-R patients. Therefore, the survival benefit is most likely due to favorable genetic background rather than therapeutic benefit from RCHOP.

In summary, we demonstrate that patients with *MYC*-EC large B-cell lymphoma show prognostic features intermediate between *MYC*-N and *MYC*-R large B-cell lymphoma. Although p53 expression is significantly associated with *MYC*-EC, *MYC*-R, and MYC expression in our study, the negative synergistic effect of p53 expression on survival only occurs

in *MYC*-R B-cell lymphoma. CD99 expression is associated with *MYC*-N B-cell lymphoma and shows positive impact on survivals, particularly in MYC and p53 double-negative patients, further supporting the heterogeneous nature of DLBCL. Precise evaluation of different biomarkers in DLBCL may shed light on new targeted therapies.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humpath.2018.11.015.

Acknowledgment

We thank Ms Sonora Thigpen for assistance with the figures.

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