Clinical features, tumor biology, and prognosis associated with *MYC* rearrangement and Myc overexpression in diffuse large B-cell lymphoma patients treated with rituximab-CHOP

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MYC dysregulation, including *MYC* gene rearrangement and Myc protein overexpression, is of increasing clinical importance in diffuse large B-cell lymphoma (DLBCL). However, the roles of *MYC* and the relative importance of rearrangement vs overexpression remain to be refined. Gaining knowledge about the tumor biology associated with *MYC* dysregulation is important to understand the roles of *MYC* and *MYC*-associated biology in lymphomagenesis. In this study, we determined *MYC* rearrangement status ($n=344$) and Myc expression ($n=535$) in a well-characterized DLBCL cohort, individually assessed the clinical and pathobiological features of patients with *MYC* rearrangement and Myc protein overexpression, and analyzed the prognosis and gene expression profiling signatures associated with these *MYC* abnormalities in germinal center B-cell-like and activated B-cell-like DLBCL. Our results showed that the prognostic importance of *MYC* rearrangement vs Myc overexpression is significantly different in germinal center B-cell-like vs activated B-cell-like DLBCL. In germinal center B-cell-like DLBCL, *MYC*-rearranged germinal center B-cell-like DLBCL patients with Myc overexpression significantly contributed to the clinical, biological, and prognostic characteristics of the overall

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Myc-overexpressing germinal center B-cell-like DLBCL group. In contrast, in activated B-cell-like DLBCL, the occurrence, clinical and biological features, and prognosis of Myc overexpression were independent of *MYC* rearrangement. High Myc levels and Myc-independent mechanisms, either tumor cell intrinsic or related to tumor microenvironment, conferred significantly worse survival to *MYC*-rearranged germinal center B-cell-like DLBCL patients, even among Myc^{high}Bcl-2^{high} DLBCL patients. This study provides new insight into the tumor biology and prognostic effects associated with *MYC* dysregulation and suggest that detection of both *MYC* translocations and evaluation of Myc and Bcl-2 expression is necessary to predict the prognosis of DLBCL patients.

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MYC is a proto-oncogene that encodes the Myc protein, which is critical for cell proliferation, growth, metabolism, differentiation, apoptosis, and immune responses. In mouse models, Myc inactivation induces sustained tumor regression via both tumor cell-intrinsic and host-dependent mechanisms.¹ The selective small-molecule bromodomain inhibitor JQ1 has a potent antiproliferative effect that was associated with the effective downregulation of *MYC* and Myc target genes² rendering this agent to have great therapeutic potential.³

The chromosomal rearrangement or translocation involving *MYC* and other genes (most commonly the immunoglobulin heavy-chain gene (*IGH*) locus) leads to Myc overexpression and occurs in ~10% of diffuse large B-cell lymphomas (DLBCLs).^{4–8} DLBCL is the most common type of non-Hodgkin's lymphoma, and among *MYC*-rearranged aggressive lymphomas, DLBCL is the entity that clinicians most commonly encounter.^{9,10} Several studies have reported that *MYC* translocations independently predicted significantly poor survival in DLBCL patients.^{11–16} However, other studies found inconsistent results^{17–19} or limitations of its prognostic significance.^{20,21}

The clinical significance of Myc overexpression in DLBCL has also been the source of much attention and controversy. Several groups including ours have found that DLBCL with high Myc protein expression detected by immunohistochemistry had inferior survival.^{5,13,22–24} Furthermore, the poor prognosis associated with Myc overexpression was contributed by cases with Myc/Bcl-2 coexpression—‘double-positive lymphoma’ (DPL)—which account for 18–44% of DLBCLs.^{13,20,23–26} However, one study showed that the prognostic value of double-positive lymphoma was lost in younger DLBCL patients with poor prognosis.²⁷ Inconsistent results have been reported regarding whether the prognostic significance of Myc or Myc/Bcl2 protein expression depends on *MYC* or *MYC/BCL2* gene rearrangement status or not.^{8,13,20} Other issues include whether Myc/Bcl2 immunohistochemistry is robust and reproducible,²⁸ and that immunohistochemistry cut-off values have varied among different study groups,^{13,20,23–26} which may affect the specificity of this combined biomarker for poorer prognosis.

Possible molecular mechanisms underlying the inconsistent clinical results may include presence or absence of other genetic abnormalities and oncogenic pathways,^{29–31} as well as another aspect of Myc function: promoting apoptosis.³² Moreover, researchers have recently reported findings that Myc is a universal amplifier of 10–15% in human genome, suggesting that Myc function is nonspecific and that the consequences of *MYC* activities are affected by pre-existing molecular programs in the tumor cells.^{33,34} Therefore, tumor biology associated with *MYC*/Myc (designated *MYC* herein) abnormalities may have important roles in the observed adverse prognosis.

Taken together, both *MYC* gene rearrangement and Myc protein overexpression have been correlated with significantly adverse prognosis in DLBCL. However, how much of these two biomarkers overlap and differ, how much their associated tumor biology affects the prognostic effects, and whether the *MYC* functional role is molecular context-dependent are not very clear. In this study, we compared the occurrence and clinicopathologic features of patients stratified by *MYC* rearrangement and Myc expression status, and analyzed the differential prognosis and gene expression profiling associated with these *MYC* abnormalities in a well-characterized DLBCL cohort to assess the utility of these two genetic and protein biomarkers and explore the prognostic determinants. This study is important for achieving the goal of precision medicine in DLBCL.

Patients and methods

Patients

The study cohort consisted of 539 R-CHOP-treated patients with *de novo* DLBCL from the International DLBCL Rituximab-CHOP Consortium Program, including 466 cases from the training set of a previous study,²⁴ and additional 73 cases with either Myc immunohistochemistry or *MYC* gene rearrangement status determined. The diagnostic criteria, review process, and eligibility and exclusion criteria have been described previously.^{35,36} The cell-of-origin classification as either the germinal center

B-cell-like (GCB) or activated B-cell-like (ABC) subtype was determined using gene expression profiling and/or immunohistochemistry for CD10, BCL6, GCET-1, FOXP1, and MUM1 using the Visco-Young and/or Choi algorithms as described previously.^{24,35,36} Totally, 276 cases were classified as GCB, 259 cases were classified as ABC, and 4 cases were unclassifiable. All patients underwent standard R-CHOP or R-CHOP-like therapy, and the median follow-up time was 45 months (range, 30–176.1 months). This study was conducted in accordance with the Declaration of Helsinki and was approved as being of minimal to no risk or as exempt by the institutional review boards of all participating centers, including The University of Texas MD Anderson Cancer Center.

Fluorescence *In Situ* Hybridization, Immunohistochemistry, and Gene Sequencing

MYC translocation was detected by fluorescence *in situ* hybridization using two probes (a locus-specific identifier *IGH/MYC/CEP8* tri-color dual-fusion probe and a locus-specific identifier *MYC* dual-color break-apart probe) ($n=344$). Myc expression was assessed by immunohistochemistry using tissue microarray sections and a monoclonal anti-(c) MYC antibody, clone Y69 (Epitomics, Burlingame, CA, USA) ($n=535$). The experimental techniques and scoring processes have been described previously.^{15,21,24}

Evaluation of other biomarker expression by immunohistochemistry was also performed on tissue microarray sections using corresponding antibodies: p53 (DO-7; Dako, Carpinteria, CA, USA), MDM2 (IF2; Calbiochem, Billerica, MA, USA), Bcl-2 (Clone-124; Dako), Ki-67 (MIB-1; Dako), pAKT (726E11; CST), Bcl-6 (PG-B6p; DAKO), FOXP1 (EPR4113; Abcam), MUM1/IRF4 (Dako), CD10 (56C6; Vantana), CD30 (clone BerH2; Dako), BLIMP-1 (EPR16655; Epitomics), NF- κ B subunits (Dako), CXCR4 (Abcam, Cambridge, MA, USA), and survivin (EP2880Y; Epitomics). *BCL6* and *BCL2* translocations and *TP53* mutations were detected as described previously.^{15,21,24,35–42}

Gene Expression Profiling

Gene expression profiling for 457 patients was performed using the Affymetrix GeneChip Human Genome HG-U133 Plus 2.0 Array as described previously.^{21,24,35–37} The CEL files have been deposited in the National Center for Biotechnology Information Gene Expression Omnibus repository (GSE31312). Microarray data were normalized for further supervised clustering analysis. Multiple *t*-tests were used to identify differentially expressed genes and the *P*-values obtained were corrected for the false discovery rate using the β -uniform mixture method.

Statistical Analysis

The clinical and pathobiological features of DLBCL patients at the time of presentation were compared using the χ^2 test. The mean expression levels of biomarkers between DLBCL groups with or without *MYC* abnormalities were also compared by unpaired *t*-test. Overall survival (OS) was calculated from the time of diagnosis to last follow-up or death from any cause. Progression-free survival (PFS) was calculated from the time of diagnosis to disease progression, disease relapse, or death from any cause. Patients who were alive and disease progression-free at last follow-up were censored. Survival analysis was performed using the Kaplan–Meier method with the Prism 5 program (GraphPad Software, San Diego, CA, USA), and differences in survival were compared using the log-rank (Mantel–Cox) test. Multivariate survival analysis was performed using a Cox proportional hazards regression model with the SPSS software program (version 19.0; IBM Corporation, Armonk, NY, USA). All differences with $P \leq 0.05$ were considered statistically significant.

Results

Myc Expression in Germinal Center B-Cell-Like and Activated-B-Cell-Like Subtypes of DLBCL

We observed variable levels of nuclear Myc expression in DLBCL ($n=535$) (Figures 1a–c). The mean expression level of Myc in ABC-DLBCL was significantly higher compared with that in GCB-DLBCL (Figure 1d). Among the cases successfully tested for *MYC* rearrangement status ($n=344$), 27 (16.3%) of 166 GCB-DLBCL cases and 13 (7.3%) of 177 ABC-DLBCL cases had *MYC* rearrangement/translocation. Most but not all *MYC*-rearranged cases (*MYC*-R⁺) had high *MYC*-mRNA and Myc expression, with an average percentage of Myc^{high} nuclei in the tumor samples of 70.5% (Supplementary Figure S1A). We thus set the cutoff for Myc overexpression (Myc^{high}) at $\geq 70\%$, so that Myc expression levels in *MYC*-non-rearranged (*MYC*-R[−]) Myc^{high} patients comparably ‘matched’ those in *MYC*-R⁺/Myc^{high} cases (Supplementary Figure S1C) in comparisons of clinical and biological significance of Myc overexpression vs *MYC* translocation.

Using this cutoff, we found that 175 (32.7%) of 535 DLBCL patients, including 76 (27.9%) of 272 GCB-DLBCL patients and 98 (37.8%) of 259 ABC-DLBCL patients, were Myc^{high} (Figures 2a and b). Nineteen (73%) of the 26 *MYC*-R⁺ GCB patients (one *MYC*-R⁺ GCB case had no expression data available) and 7 (54%) of the 13 *MYC*-R⁺ ABC patients were Myc^{high}, who had significantly higher *MYC* transcripts and significantly worse survival compared with the *MYC*-R⁺/Myc^{low} cases (Figures 1e–g and Supplementary Figures S1D). Compared with *MYC*-R[−] patients, *MYC*-R⁺/Myc^{high} patients had significantly worse survival in GCB-DLBCL ($P=0.0001$ for OS and

Figure 1 (a–c) Histograms of Myc expression according to immunohistochemistry staining in all diffuse large B-cell lymphoma (DLBCL) patients, germinal center B-cell-like (GCB) DLBCL patients, and activated B-cell-like (ABC) DLBCL patients. (d) The mean Myc protein levels of ABC-DLBCL were significantly higher compared with those of GCB-DLBCL. (e and f) MYC rearrangement-positive (MYC-R⁺) DLBCL without high Myc immunohistochemistry scores (either GCB or ABC subtype) had significantly lower MYC-mRNA levels. (g) MYC-R⁺ DLBCL patients without high Myc immunohistochemistry scores had significantly better overall survival compared with MYC-R⁺/Myc^{high} DLBCL. Myc^{high}, high Myc protein expression; Myc^{low}, low Myc protein expression.

$P < 0.0001$ for PFS) but not in ABC-DLBCL ($P = 0.56$ for OS and $P = 0.14$ for PFS) (Supplementary Figures S1E and F). Comparison of MYC expression levels between the MYC-R⁺ GCB, MYC-R⁻ GCB, MYC-R⁺ ABC, and MYC-R⁻ ABC Myc^{high} groups revealed that MYC-R⁺/Myc^{high} GCB-DLBCL had significantly higher Myc protein expression levels compared with all other subsets, and that among both the GCB and ABC subtypes, MYC-R⁺/Myc^{high} cases had a significantly higher level of MYC-mRNA levels compared with MYC-R⁻/Myc^{high} cases (Figures 2c and d).

Clinical Parameters Associated with Myc Overexpression and MYC Rearrangement

We analyzed the clinical parameters associated with Myc overexpression (Table 1) and MYC rearrangement (Supplementary Table 1). We further compared MYC-R⁺/Myc^{high} and MYC-R⁻/Myc^{high} cases (Supplementary Table S2). Results of significant characteristics are summarized in Supplementary Table S3. Largely, both Myc overexpression and MYC rearrangement could identify a subgroup of patients with adverse clinical features in GCB-DLBCL, whereas patients with these abnormalities did not show such distinct characteristics among ABC-DLBCLs.

For example, in GCB-DLBCL, Myc^{high} patients compared with Myc^{low} patients more often had stage III/IV disease, ≥ 2 extranodal sites, ECOG performance status ≥ 2 , tumor size ≥ 5 cm, International Prognostic Index > 2 , bone marrow involvement at clinical presentation, and less likely to have complete response. In ABC-DLBCL, Myc^{high} patients compared with Myc^{low} patients had a higher proportion of women, and did not show significant association with other clinical parameters. The GCB and ABC subtypes of Myc^{high} patients differed significantly only in their frequencies of patients with age ≥ 60 years (Table 1). Similarly, in GCB-DLBCL, MYC-R⁺ patients were enriched in patients with adverse clinical features, whereas in ABC-DLBCL, MYC rearrangement was only associated with primary extranodal origin. The GCB subtype compared with the ABC subtype of MYC-R⁺ patients showed trends toward having a higher proportion of patients with bone marrow involvement and non-complete treatment response (Supplementary Table S1).

In both the GCB- and ABC-DLBCL groups, MYC-R⁺/Myc^{high} patients appeared to have similar clinical features with MYC-R⁻/Myc^{high} patients (except complete response rate), yet may have elevated serum lactate dehydrogenase levels and higher frequency of extranodal origin (Supplementary Table S2).

Figure 2 Occurrence of *MYC* rearrangement (*MYC*-R) and overexpression (*Myc*^{high}) and *MYC* expression levels in diffuse large B-cell lymphoma (DLBCL). (a and b) Schematic diagrams showing the frequencies of *MYC* translocation (*MYC*-R⁺) and *Myc* overexpression (*Myc*^{high}) and their overlaps in germinal center B-cell-like (GCB) and activated B-cell-like (ABC) DLBCL in the current study cohort. (c and d) Comparisons of *Myc* protein and *MYC*-mRNA expression levels in DLBCL patients with or without *MYC* dysregulation. Among *Myc*^{high} groups, *MYC*-R⁺ GCB-DLBCL had highest *Myc* protein levels; *MYC*-R⁺ DLBCL (both GCB and ABC subtypes) had significantly higher *MYC*-mRNA levels compared with *MYC*-R⁻ DLBCL. Note: Each dot represents one patient in the study cohort. *MYC*-R⁺, *MYC* rearrangement-positive; *MYC*-R⁻, *MYC* rearrangement-negative; *Myc*^{high}, high *Myc* protein expression/*Myc* overexpressing; *Myc*^{low}, low *Myc* protein expression; *MYC*-R^{N/A}, *MYC* rearrangement status not available.

Molecular Biomarkers Associated with *Myc* Overexpression and *MYC* Rearrangement

Myc^{high} DLBCL compared with *Myc*^{low} DLBCL, in addition to having higher *Myc* levels and significantly higher frequencies of *MYC* translocation and ABC subtype (Table 1), had higher expression levels of p53, MDM2, Bcl-2 (*Myc*^{high} ABC-DLBCL only), Bcl-6, FOXP1, IRF4/MUM1 (*Myc*^{high} ABC-DLBCL only), Ki-67, pAKT, CXCR4, and CD10 (*Myc*^{high} GCB-DLBCL only), but lower expression levels of BLIMP-1 (in ABC-DLBCL only) and nuclear expression of the NF- κ B subunits c-Rel and RelB (in ABC-DLBCL only, $P=0.019$, figure not shown) (Figure 3). At the mRNA level, only *MDM2* (in GCB-DLBCL only), *FOXP1* (in ABC-DLBCL only), *IRF4* (in ABC-DLBCL only), and *MME/CD10* transcript levels showed corresponding correlations consistent with those at the protein level (Figures 4a–d). *PRDM1* mRNA levels were significantly lower in *Myc*^{high} GCB-DLBCL compared with that in *Myc*^{low} GCB-DLBCL ($P=0.0005$). The GCB and ABC subtypes of *Myc*^{high} patients differed in frequencies of *MYC* translocation, *BCL2* translocation, *TP53* mutation (all

higher in the GCB subtype), and expression levels of Bcl-2, p50, c-Rel (all higher in the ABC subtype), pAKT (higher in the GCB subtype), and cell-of-origin-related biomarkers (Bcl-6, BLIMP-1, GCET-1, CD10, FOXP1, MUM1) (Table 1, Figure 3, and Supplementary Table S3).

Some of the pathobiological associations with *Myc*^{high} DLBCL were shared by *MYC*-R⁺ GCB-DLBCL patients (compared with *MYC*-R⁻ GCB-DLBCL; Supplementary Tables S1 and S3). On the other hand, in addition to the difference in *Myc* activation mechanisms (*MYC* translocation or not), *MYC*-R⁺/*Myc*^{high} compared with *MYC*-R⁻/*Myc*^{high} patients had higher frequencies of GCB subtype and *BCL2* translocation, and lower frequencies of *BCL6* translocation, MUM1, CD30, and p52 expression (Supplementary Table S2 and S3). Among GCB-DLBCL patients, *MYC*-R⁺/*Myc*^{high} compared with *MYC*-R⁻/*Myc*^{high} patients had significantly higher expression levels of MDM2, CD10, and FOXP1 but a lower expression level of CD30; among ABC-DLBCL patients, *MYC*-R⁺/*Myc*^{high} compared with *MYC*-R⁻/*Myc*^{high} was associated with lower expression levels of Bcl-6 and pAKT (Figures 4f–l).

Table 1 Clinicopathologic features of DLBCL^a

Variables	DLBCL		<i>P</i> ₁ values	GCB-DLBCL		<i>P</i> ₂ values	ABC-DLBCL		<i>P</i> ₃ values	<i>P</i> ₄ values
	<i>Myc</i> ^{high} N (%)	<i>Myc</i> ^{low} N (%)		<i>Myc</i> ^{high} N (%)	<i>Myc</i> ^{low} N (%)		<i>Myc</i> ^{high} N (%)	<i>Myc</i> ^{low} N (%)		
Variables	175 (100)	360 (100)		76 (100)	196 (100)		98 (100)	161 (100)		0.016
<i>Age (years)</i>										
< 60	73 (41.7)	150 (41.7)	1.0	42 (55.3)	91 (46.4)	0.22	30 (30.6)	57 (35.4)	0.50	0.0012
≥ 60	102 (58.3)	210 (58.3)		34 (44.7)	105 (53.6)		68 (69.4)	104 (64.6)		
<i>Gender</i>										
Female	78 (44.6)	146 (40.6)	0.38	30 (39.5)	85 (43.4)	0.56	48 (49.0)	59 (36.6)	0.05	0.21
Male	97 (55.4)	214 (59.4)		46 (60.5)	111 (56.6)		50 (51.0)	102 (63.4)		
<i>Stage</i>										
I and II	64 (37.9)	179 (51.3)	0.0041	31 (41.9)	110 (58.5)	0.015	32 (34.0)	68 (43.0)	0.16	0.30
III and IV	105 (62.1)	170 (48.7)		43 (58.1)	78 (41.5)		62 (66.0)	90 (57.0)		
<i>B-symptoms</i>										
No	103 (62.4)	227 (66.2)	0.41	45 (63.4)	133 (71.9)	0.19	58 (62.4)	91 (58.7)	0.57	0.89
Yes	62 (37.6)	116 (33.8)		26 (36.6)	52 (28.1)		35 (37.6)	64 (41.3)		
<i>LDH level</i>										
Normal	58 (35.4)	137 (41.6)	0.18	24 (34.3)	82 (46.1)	0.091	34 (36.6)	54 (36.5)	0.099	0.76
Elevated	106 (64.6)	192 (58.4)		46 (65.7)	96 (53.9)		59 (63.4)	94 (63.5)		
<i>Number of extranodal sites</i>										
0–1	117 (69.6)	279 (80.9)	0.0045	45 (63.4)	156 (83.9)	0.0004	67 (69.8)	120 (76.9)	0.21	0.38
≥ 2	51 (30.4)	66 (19.1)		26 (36.6)	30 (16.1)		29 (30.2)	36 (23.1)		
<i>ECOG performance status score</i>										
0–1	126 (77.3)	275 (86.8)	0.0082	53 (76.8)	150 (89.3)	0.013	72 (77.4)	122 (83.6)	0.24	0.93
≥ 2	37 (22.7)	42 (13.2)		16 (23.2)	18 (10.7)		21 (22.6)	24 (16.4)		
<i>Size of largest tumor (cm)</i>										
< 5	64 (48.5)	170 (63.7)	0.0038	26 (47.3)	94 (64.4)	0.028	38 (49.4)	74 (62.2)	0.076	0.81
≥ 5	68 (51.5)	97 (36.3)		29 (52.7)	52 (35.6)		39 (50.6)	45 (37.8)		
<i>IPI score</i>										
0–2	88 (51.5)	234 (67.4)	0.0004	39 (52.7)	139 (74.3)	0.0007	47 (49.0)	92 (58.6)	0.13	0.63
3–5	83 (48.5)	113 (32.6)		35 (47.3)	48 (25.7)		49 (51.0)	65 (41.4)		
<i>Therapy response</i>										
CR	117 (66.9)	285 (79.2)	0.002	46 (60.5)	155 (79.1)	0.0018	70 (71.4)	127 (78.9)	0.17	0.13
PR	33	38		24	22		21	16		
SD	9	15		5	10		4	5		
PD	16	22		1	9		3	13		
<i>Primary origin</i>										
LN DLBCL	111 (63.8)	227 (63.9)	0.97	47 (63.5)	125 (64.8)	0.85	61 (63.5)	100 (62.9)	0.92	1.00
EN DLBCL	63 (36.2)	128 (36.1)		27 (36.5)	68 (35.2)		35 (36.5)	59 (37.1)		
<i>Bone marrow involvement</i>										
No	130 (83.9)	287 (92.6)	0.0036	52 (81.3)	163 (95.3)	0.0006	77 (85.6)	122 (89.1)	0.43	0.48
Yes	25 (16.1)	23 (7.4)		12 (18.8)	8 (4.7)		13 (14.4)	15 (10.9)		
<i>Ki-67 index (%)</i>										
< 70	40 (22.9)	149 (42.2)	< 0.0001	24 (31.6)	83 (43.2)	0.097	16 (16.3)	65 (40.6)	< 0.0001	0.028
≥ 70	135 (77.1)	204 (57.8)		52 (68.4)	109 (56.8)		82 (83.7)	95 (59.4)		
<i>TP53 mutations</i>										
No	114 (74.5)	252 (78.8)	0.35	43 (65.2)	131 (75.7)	0.10	71 (81.6)	118 (81.9)	0.95	0.02
Yes	39 (25.5)	68 (21.3)		23 (34.8)	42 (24.3)		16 (18.4)	26 (18.1)		
<i>MYC translocation</i>										
No	81 (75.7)	220 (94.4)	< 0.0001	23 (54.8)	113 (94.2)	< 0.0001	58 (89.2)	106 (94.6)	0.23	< 0.0001
Yes	26 (24.3)	13 (5.6)		19 (45.2)	7 (5.8)		7 (10.8)	6 (5.4)		
<i>BCL2 translocation</i>										
No	121 (86.4)	230 (79.9)	0.11	38 (67.9)	106 (67.9)	1.00	83 (98.8)	123 (93.9)	0.09	< 0.0001
Yes	19 (13.6)	58 (20.1)		18 (32.1)	50 (32.1)		1 (1.2)	8 (6.1)		
<i>BCL6 translocation</i>										
No	77 (64.2)	169 (68.7)	0.38	37 (71.2)	103 (75.7)	0.52	39 (58.2)	65 (59.6)	0.85	0.14
Yes	43 (35.8)	77 (31.3)		15 (28.8)	33 (24.3)		28 (41.8)	44 (40.4)		
<i>Bcl-2 expression</i>										
< 70%	76 (43.7)	195 (55.7)	0.012	40 (53.3)	121 (63.4)	0.16	35 (35.7)	73 (46.2)	0.12	0.03
≥ 70%	98 (56.3)	155 (44.3)		35 (46.7)	70 (36.6)		63 (64.3)	85 (53.8)		

Abbreviations: ABC, activated-B-cell-like; CR, complete response; DLBCL, diffuse large B-cell lymphoma; ECOG, Eastern Cooperative Oncology Group; EN, extranodal; GCB, germinal-center-B-cell-like; IPI, International Prognostic Index; LDH, lactate dehydrogenase; LN, lymph node; *Myc*^{high}, high *Myc* protein expression; *Myc*^{low}, low *Myc* protein expression; PD, progressive disease; PR, partial response; SD, stable disease.

Note: *P*-values indicate the significance of differences in the positivity frequencies of the listed parameters between two groups. *P*₁ values are for comparisons between overall *Myc*^{high} and *Myc*^{low} DLBCL patients; *P*₂ values are for comparisons between *Myc*^{high} and *Myc*^{low} GCB-DLBCL patients; *P*₃ values are for comparisons between *Myc*^{high} and *Myc*^{low} ABC-DLBCL patients; and *P*₄ values are for comparisons between *Myc*^{high} GCB-DLBCL and *Myc*^{high} ABC-DLBCL patients. For therapy response, we calculated *P*-values for differences between CR and other responses.

^aWith high or low *Myc* expression levels (*Myc*^{high} vs *Myc*^{low}) in the overall, GCB, and ABC cohorts. Bold values are statistically significant.

Prognostic Effect of Myc Overexpression with or without MYC Translocations and Associated Gene Expression Profiling Signatures

We combined the survival analysis with gene expression profiling comparisons to assess the role of MYC abnormalities in DLBCL and to study potential mechanisms. As in earlier studies,^{13,22–24} in the present study, high expression levels of Myc was associated with significantly worse OS and PFS in both GCB- and ABC-DLBCL (Supplementary Figure S2). Similar prognostic impact was also shown by high MYC-mRNA levels in GCB- and ABC-DLBCL (Supplementary Figures S3A–D). Gene expression profiling analysis showed that Myc^{high} GCB- and ABC-DLBCL had different gene expression profiling signatures (Table 2, Supplementary Figures S2D and F, and Supplementary Table S4A). The significantly differentially expressed genes between these two groups included *CDCA7L*, *IGF2BP3*, and *RUVBL2*, which are known to have roles in the oncogenic transformation by MYC or to interact with MYC.

MYC rearrangement did not show prognostic significance in the Myc^{low} cases [for OS: $P=0.25$ (OS of MYC-R⁺/Myc^{low} patients was slightly better than MYC-R⁻/Myc^{low} DLBCL); for PFS: $P=0.71$]. Dividing the Myc^{high} cases with unfavorable prognosis into MYC-R⁺/Myc^{high} and MYC-R⁻/Myc^{high} two types and comparing their prognosis and gene expression profiling features, we further found that MYC-R⁺/Myc^{high} GCB-DLBCL and MYC-R⁻/Myc^{high} ABC-DLBCL were the main contributors to the overall worse prognosis and distinct gene expression profiling signatures of the Myc^{high} GCB-DLBCL and Myc^{high} ABC-DLBCL groups, respectively.

In the GCB-DLBCL group, MYC-R⁺/Myc^{high} patients had significantly worse survival than both Myc^{low} and MYC-R⁻/Myc^{high} patients did. Although MYC-R⁻/Myc^{high} GCB-DLBCL showed trends towards worse survival compared with the overall Myc^{low} GCB-DLBCL ($P=0.40$ for OS, Figure 5a; $P=0.48$ for PFS; Supplementary Figure S4A), and the MYC-R⁻/Myc^{low} GCB-DLBCL (for OS: $P=0.32$; Supplementary Figure S4C; for PFS: $P=0.21$), the differences were not significant. Biologically, only MYC-R⁺/Myc^{high} (but not MYC-R⁻/Myc^{high}) GCB-DLBCL compared with Myc^{low} GCB-DLBCL showed a distinct gene expression profiling signature (false discovery rate <0.01) (Figure 5b, Supplementary Table S4B, and Table 3), and this signature mostly overlapped the Myc^{high} gene expression profiling signature identified in the overall GCB-DLBCL group (Table 2 and Supplementary Figure S2D) involving cell proliferation, gene expression, metabolism, apoptosis, microenvironment and immune response, and microRNA genes. In contrast, the MYC-R⁻/Myc^{high} GCB-DLBCL patients compared with Myc^{low} GCB-DLBCL (Supplementary Figures S4D, Table 3, and Supplementary Table S4B) or MYC-R⁺/Myc^{high} GCB-DLBCL (Figure 5c, Table 3,

and Supplementary Table S4B) only showed a few differentially expressed genes below false discovery rate threshold of 0.30 (not including MYC), most of which are involved in cell proliferation, gene expression, ribosome biogenesis, and metabolism, suggesting the presence of heterogeneity and post-transcriptional regulation of MYC as a cause of Myc overexpression within the MYC-R⁻/Myc^{high} GCB-DLBCL group. The comparison between MYC-R⁺ GCB-DLBCL and MYC-R⁻ GCB-DLBCL overall is shown in Supplementary Figure S4E.

In contrast, in ABC-DLBCL, the prognostic significance (Figure 5d) and gene expression profiling features of Myc overexpression did not depend on MYC translocation. MYC-R⁻/Myc^{high} ABC-DLBCL had significantly poorer survival compared with the overall Myc^{low} or MYC-R⁻/Myc^{low} ABC-DLBCL (Figure 5d and Supplementary Figures S4G–I) and distinct gene expression profiling signatures (false discovery rate <0.01 ; Figure 5e and Supplementary Table S4C). The genes in the MYC-R⁻/Myc^{high} ABC-DLBCL gene expression profiling signature, overlapped with differentially expressed genes between the overall Myc^{high} vs Myc^{low} ABC-DLBCL patients, included typical Myc targets mainly related to cell proliferation, the cell cycle, gene expression, ribosome biogenesis, metabolism (Table 4), and cooperating oncogenes such as *RUVBL2*, as well as *IGF2BP3* involved in post-transcriptional regulation of MYC, and *HINT1* modulating p53 levels and the p53 pathway. Different from the MYC-R⁻/Myc^{high} ABC-DLBCL cases, MYC-R⁺/Myc^{high} ABC-DLBCL compared with overall Myc^{low} ABC-DLBCL only showed nonsignificant trends towards worse OS and PFS (Figure 5d and Supplementary Figures S4G–I) and only a few differentially expressed genes (false discovery rate <0.30 , Supplementary Figure S4F). No genes were found differentially expressed between MYC-R⁺/Myc^{high} ABC-DLBCL and MYC-R⁻/Myc^{high} ABC-DLBCL, or between MYC-R⁺/Myc^{high} ABC-DLBCL and the overall MYC-R⁻ ABC-DLBCL group. This is in contrast with the distinct gene expression profiling feature of MYC-R⁺/Myc^{high} GCB-DLBCL shown in Figure 5b and Supplementary Figures S4E. Comparison between overall MYC-R⁺/Myc^{high} ABC-DLBCL and MYC-R⁺/Myc^{high} GCB-DLBCL indicated their different and potentially heterogeneous tumor biology (Figure 5f) (between MYC-R⁺/Myc^{high} and MYC-R⁺/Myc^{low} GCB-DLBCL, or between MYC-R⁺/Myc^{high} and MYC-R⁺/Myc^{low} ABC-DLBCL, we did not find significant differentially expressed genes below false discovery rate thresholds of 0.05–0.50). MYC-R⁺ ABC-DLBCL appears to have decreased B-cell receptor signaling compared with MYC-R⁺ GCB-DLBCL (false discovery rate <0.30 ; Table 4 and Supplementary Table S4C). However, the ‘loss’ of the gene expression profiling signature may also be due to the small case number.

Comparing between GCB and ABC subtypes of Myc^{high} patients, the overall GCB and ABC subtypes

Figure 3 Expression of pathobiological markers in germinal center B-cell-like (GCB) and activated B-cell-like (ABC) diffuse large B-cell lymphoma with or without Myc overexpression. In both GCB- and ABC-DLBCL, Myc overexpression was associated with significantly higher levels of p53 (a), MDM2 (b), Ki-67 (d), Bcl-6 (f), and FOXP1 (g), and a significantly lower level of c-Rel (k). In GCB-DLBCL only, Myc overexpression was associated with significantly higher levels of CD10 (e), pAKT (j), and CXCR4 (l). In ABC-DLBCL only, Myc overexpression was associated with significantly higher levels of Bcl-2 (c) and MUM1 (h) levels and a significantly lower level of BLIMP-1 (i). Myc^{high}, high Myc protein expression; Myc^{low}, low Myc protein expression.

of Myc^{high} patients showed no significant difference in survival (ABC subtype showed nonsignificant trends towards poorer survival; Supplementary Figures S3E and F). However, MYC-R⁺/Myc^{high} GCB-DLBCL showed unfavorable trends compared with Myc^{high} ABC-DLBCL (either MYC-R⁻ or MYC-R⁺). The P-value for the difference in PFS between the MYC-R⁺/Myc^{high} GCB-DLBCL and MYC-R⁻/Myc^{high} ABC-DLBCL patients was 0.058 (Figure 5g). MYC-R⁺/Myc^{high} GCB-DLBCL compared with MYC-R⁻/Myc^{high} ABC-DLBCL or Myc^{low} ABC-DLBCL showed distinct gene expression profiling signatures overlapping with the one comparing with MYC-R⁻ GCB-DLBCL. Comparisons between MYC-R⁺ DLBCL and MYC-R⁻ DLBCL overall (regardless of Myc^{high} or Myc^{low}, GCB or ABC), and between MYC-R⁺/Myc^{high} DLBCL overall and MYC-R⁻/Myc^{high} DLBCL overall (regardless of GCB or ABC) are shown in Figures 5h and i (Table 5 and Supplementary Table S4D). These analyses suggest the distinctive biology of MYC-R⁺/Myc^{high} GCB-DLBCL and cell of origin during

lymphomagenesis may have a role in defining its biological feature.

Concurrent Evaluation of Myc/Bcl-2 Overexpression and MYC Translocations

We examined whether the prognostic value of Myc overexpression is contributed by or depends on the molecular marker associations with Myc^{high} DLBCL as shown in Figure 3. As shown previously, the prognostic significance of Myc^{high} and Bcl-2^{high} in DLBCL significantly depend on each other.^{13,20,23–26} In addition, to a certain extent, the prognostic significance of Myc^{high} showed dependence on CXCR4,⁴⁰ FOXP1, and MUM1 overexpression, which are also associated with Bcl-2 overexpression (the association of MUM1 was only in ABC but not in GCB), high Ki-67 (for OS but not for PFS), and low BLIMP-1 expression (Supplementary Figure S5).

Figure 4 (a–d) Myc overexpression was correlated with significantly higher levels of *MDM2*, *IRF4*, and *MME/CD10* mRNA expression in germinal center B-cell-like (GCB) diffuse large B-cell lymphoma and significantly higher levels of *FOXP1* and *IRF4* mRNA expression in activated B-cell-like (ABC) diffuse large B-cell lymphoma (DLBCL). (e) The *MYC*-R⁺/*MYC*^{high} group had significantly lower levels of MUM1 expression. (f–j) The *MDM2*, *CD30*, *CD10*, and *FOXP1* levels in *MYC*^{high} GCB-DLBCL patients with *MYC* translocation were significantly different from those of *MYC*^{high} GCB-DLBCL patients without *MYC* translocation. (k and l) The *Bcl-6* and *pAKT* levels of *MYC*^{high} ABC-DLBCL patients with *MYC* translocation were significantly lower compared with those of *MYC*^{high} ABC-DLBCL patients without *MYC* translocation. Myc^{high}, high Myc protein expression; Myc^{low}, low Myc protein expression; MYC-R⁺, MYC rearrangement-positive; MYC-R⁻, MYC rearrangement-negative; MYC-R^{N/A}, MYC rearrangement status not available.

Table 2 Gene expression profile signatures of Myc protein overexpression in DLBCL, GCB-DLBCL, and ABC-DLBCL

	DLBCL	GCB-DLBCL	ABC-DLBCL
	<i>Myc</i> ^{high} vs <i>Myc</i> ^{low} (false discovery rate < 0.05, fold change > 1.53)	<i>Myc</i> ^{high} vs <i>Myc</i> ^{low} (false discovery rate < 0.01, fold change > 1.68)	<i>Myc</i> ^{high} vs <i>Myc</i> ^{low} (false discovery rate < 0.01, fold change > 1.31)
Upregulated	<i>MYC</i> , <i>AICDA</i> , <i>SNHG1</i> , <i>SNHG4</i> , <i>TMEM97</i> , <i>PAICS</i> , <i>TCL1A</i> , <i>XK</i> , <i>FAM129C</i> , <i>CKS2</i> , <i>PEG10</i> , <i>IGF2BP3</i> , <i>SLC16A1</i> , <i>FAM72A</i> /B/C/D, <i>HELLS</i> , <i>CDCA7L</i> , <i>MAD2L1</i> , <i>MRPL3</i> , <i>PRO2964</i> , <i>C13orf18</i> , <i>MIR17HG</i>	<i>MYC</i> , <i>PEG10</i> , <i>SNHG4</i> , <i>STRBP</i> , <i>CYP39A1</i> , <i>DKFZp686O24166</i> , <i>PAICS</i> , <i>FAM72A</i> /B/C/D, <i>CDC25A</i> , <i>SLC16A1</i> , <i>RPS21</i> , <i>DEPDC1</i> , <i>HSPD1</i>	<i>MYC</i> , <i>MAD2L1</i> , <i>EEF1E1</i> , <i>RUVBL2</i> , <i>SNHG1</i> , <i>RG9MTD1</i> , <i>MRPL3</i> , <i>IPO7</i> , <i>CCDC86</i> , <i>TFAM</i> , <i>GAR1</i> , <i>MATR3</i> , <i>SNHG4</i> , <i>TOMM5</i> , <i>NOC3L</i> , <i>WDR43</i> , <i>DDX21</i> , <i>LYAR</i> , <i>RPL24</i> , <i>LOC388796</i> , <i>SNHG8</i> , <i>WDR75</i> , <i>DCTPP1</i> , <i>MAT2A</i> , <i>QDPR</i> , <i>APEX1</i>
Downregulated	<i>CD3E</i> , <i>HOPX</i> , <i>TRBC1</i> , <i>COL3A1</i> , <i>RGS1</i> , <i>COL3A1</i> , <i>TRBC1</i> , <i>ITM2A</i> , <i>GZMK</i> , <i>ITGB5</i> , <i>ITM2A</i>	<i>MIR155HG</i> , <i>TRBC1</i> , <i>GABBR1/UBD</i> , <i>CD58</i> , <i>CD3E</i> , <i>BHLHE41</i> , <i>GZMK</i> , <i>DUSP4</i> , <i>TRBC1</i> , <i>SLAMF7</i> , <i>LCP2</i> , <i>RGS1</i> , <i>ITM2A</i> , <i>SKI</i> , <i>SLAMF8</i> , <i>CD44</i> , <i>LOC285628</i> , <i>FYB</i> , <i>CCL5</i> , <i>MDFIC</i> , <i>CCND2</i> , <i>BCL11B</i> , <i>TNFAIP3</i> , <i>SLFN5</i> , <i>SNX9</i> , <i>IL10RA</i> , <i>GBP2</i>	

Abbreviations: ABC, activated B-cell-like; DLBCL, diffuse large B-cell lymphoma; GCB, germinal center B-cell-like; Myc^{high}, high Myc protein expression; Myc^{low}, low Myc protein expression.

Note: Genes are listed by the order of fold change (high to low).

Figure 5 Combined prognostic and biologic analysis of germinal-center-B-cell-like (GCB) and activated-B-cell-like (ABC) diffuse large B-cell lymphoma (DLBCL) with or without *MYC* translocation (*MYC-R*) and/or *Myc* overexpression (*Myc*^{high}) in the current study cohort. (a) GCB-DLBCL patients with both *MYC* translocation and *Myc* overexpression (*MYC-R*⁺/*MYC*^{high}) had significantly worse overall survival compared with GCB-DLBCL patients with low *Myc* expression (*Myc*^{low}) and *Myc*^{high} patients without *MYC* translocation (*MYC-R*⁻/*MYC*^{high}). The *MYC-R*⁻/*MYC*^{high} group did not have significant poorer survival compared with the *Myc*^{low} group. (b) Genes significantly differentially expressed between *MYC-R*⁺/*MYC*^{high} GCB-DLBCL patients and *Myc*^{low} GCB-DLBCL patients (false discovery rate < 0.01, fold change > 2.38). (c) Genes significantly differentially expressed between *MYC-R*⁺/*MYC*^{high} and *MYC-R*⁻/*MYC*^{high} GCB-DLBCL patients (false discovery rate < 0.30). (d) Only *MYC-R*⁻/*MYC*^{high} but not *MYC-R*⁺/*MYC*^{high} ABC-DLBCL patients had significantly OS compared with *Myc*^{low} ABC-DLBCL patients. (e) Genes significantly differentially expressed between *MYC-R*⁻/*MYC*^{high} ABC-DLBCL and *Myc*^{low} ABC-DLBCL (false discovery rate < 0.01, fold change > 1.57). (f) Genes significantly differentially expressed between ABC and GCB subtypes of *MYC-R*⁺ DLBCL. (g) *MYC-R*⁺/*MYC*^{high} GCB-DLBCL showed trend towards worse progression-free survival compared with *MYC-R*⁻/*MYC*^{high} ABC-DLBCL patients with a borderline *P*-value. (h) Genes significantly differentially expressed between *MYC-R*⁺ and *MYC-R*⁻ DLBCL (false discovery rate < 0.01, fold change > 1.66). (i) Genes significantly differentially expressed between *MYC-R*⁺/*MYC*^{high} and *MYC-R*⁻/*MYC*^{high} DLBCL (false discovery rate < 0.01, fold change > 2.05). ABC, activated-B-cell-like; GCB, germinal-center-B-cell-like; *Myc*^{high}, high *Myc* protein expression; *Myc*^{low}, low *Myc* protein expression; *MYC-R*⁺, *MYC* rearrangement-positive; *MYC-R*⁻, *MYC* rearrangement-negative.

The lack of prognostic significance of *Myc* overexpression without *MYC* translocation in GCB-DLBCL (Figure 5a) could be attributable to the favorable prognosis of *Myc*^{high}/*Bcl-2*^{low} GCB-DLBCL patients (Figures 6a and b and Supplementary Figures S6A and B). In contrast, in the ABC-DLBCL group, most *MYC-R*⁻/*MYC*^{high} patients (64%) also had *Bcl-2* overexpression, which contributed to this group's worse survival. Concurrent *Bcl-2* overexpression also had significant prognostic impact in *MYC-R*⁺/*MYC*^{high} GCB-DLBCL (Figures 6c and d), but not in overall *MYC-R*⁺ ABC-DLBCL,

MYC-R⁺/*MYC*^{high} ABC-DLBCL, or *MYC-R*⁺/*MYC*^{low} cases (Supplementary Figures S6C and D); however, we could not distinguish whether the synergy in GCB is with *Bcl-2* protein or with *BCL2* translocation. Compared with *Myc*^{low}, *MYC-R*⁻/*MYC*^{high} GCB-DLBCL, or overall *MYC-R*⁻ GCB-DLBCL, *MYC-R*⁺/*MYC*^{high} GCB-DLBCL showed nonsignificant trends toward higher *Bcl-2* levels (*P*=0.34, 0.27, and *P*=0.17, respectively; figures not shown; Supplementary Tables S1 and 2). The gene expression profiling analysis revealed no significant differentially expressed genes between *MYC-R*⁺ GCB-DLBCL

Table 3 Gene expression profile signatures of Myc protein overexpression in GCB-DLBCL

Functional categories	Upregulated	Downregulated
1. MYC-R ⁺ /Myc ^{high} vs Myc ^{low} GCB-DLBCL (false discovery rate < 0.01, fold change > 2.38)		
Signaling		TRAF1, DUSP4, GABBR1/UBD
Cell proliferation and growth, gene expression	MYC, SMAD1, BACH2, STRBP	FAM129A
Metabolism		NNMT
Cell death	PEG10, ZNF385B	BCL2A1, TMEM49
Immune response, anti-viral/anti-microbial activities	IGJ, DKFZp686O24166/ NCR3LG1	CD58, GBP1, SLAMF7, LYZ
Cell adhesion, extracellular matrix, migration		FN1, BGN, CD44
microRNAs		MIR21, MIR155HG
Unknown function	TPD52	
2. MYC-R ⁻ /Myc ^{high} vs Myc ^{low} GCB-DLBCL (false discovery rate < 0.30, fold change > 1.2)		
Signaling	RGS8, GPS1, FAM123A, PDLIM7	
Cell proliferation and growth, gene expression, ribosome biogenesis	C9orf100, SMARCA4, ZNF8, MRPS12, EMG1, INTS1	
Metabolism	SLC25A27, FADS2, ACAD9	
Microtubules, migration, cell interaction	TUBB2C, TUBB3	LGALS8
Transport	ABCA4, CHCHD4	VPS36
Long noncoding RNA, RNA gene	NAPSB	LOC202181, LOC440944
3. MYC-R ⁺ /Myc ^{high} vs MYC-R ⁻ /Myc ^{high} GCB-DLBCL (false discovery rate < 0.30, fold change > 1.3)		
Signaling	SIKE1	SPRED1
Transcription, ribosome biogenesis	NAF1, RRP1B, SMAD1	FOXN3, ATN1
Metabolism	GANC	
Extracellular matrix, migration, cytoskeleton		BGN, TRIOBP
Unknown function	PWWP2A	KIAA0913

Abbreviations: DLBCL, diffuse large B-cell lymphoma; GCB, germinal-center-B-cell-like; MYC-R⁺, MYC rearrangement-positive; MYC-R⁻, MYC rearrangement-negative; Myc^{high}, high Myc protein expression; Myc^{low}, low Myc protein expression.

Table 4 Gene expression profile signatures of Myc overexpression in ABC-DLBCL

Functional categories	Upregulated	Downregulated
1. MYC-R ⁻ /Myc ^{high} vs Myc ^{low} ABC-DLBCL (false discovery rate < 0.01, fold change > 1.57)		
Cell proliferation, cell cycle, gene expression, ribosome biogenesis	MYC, IGF2BP3, FOXP1, CCNB1, CKS2, DCAF13, THOC4, DDX11, RUVBL2, RPS15, RPLP0, RPL35, RPL27A, RPLP2, RPSA, RPS21, RPLP1, RPL15, PABPC1, DNAJC2, MAD2L1	
Metabolism	GRHPR, CYB5R2, ESD, TMEM97	
DNA damage response	EEF1E1, HINT1	
Transport	CSE1L, IPO5	
RNA gene; unknown function	SNHG1, LOC100291837	
2. MYC-R ⁺ /Myc ^{high} vs Myc ^{low} ABC-DLBCL (false discovery rate < 0.30, fold change > 1.43)		
Cell cycle, gene expression, ribosome biogenesis	MYC, RPL24, NAF1, EIF4B, CCNT1, RPL29, ZNF485	
Metabolism	GART, PLA2G12A, MDH1B	
Proteasome degradation, transport	STUB1, NXT2	
Pseudogene	RPS10P5	
3. MYC-R ⁺ ABC-DLBCL vs MYC-R ⁺ GCB-DLBCL (false discovery rate < 0.15, fold change > 1.3)		
Signaling	TNFRSF13B, SERPINA1, SH3BP5, TNIP3, ENTPD1, P2RY10, CNPY3, PGAP2	STAP1, MME
Transcription, ribosome biogenesis	BATF, MND4, RUNX1	MYBL1
Metabolism	C6orf150, KIAA0467	
Extracellular matrix, migration, cytoskeleton	IQGAP2, ARPC5, TMSB10, ACTA2, PARVB	MARCKSL1
Immune response	LILRB1/2, CD47	CAMP
Transport, degradation	NXT2, EXOC4, PTPN1	
Unknown function	GRAMD1B, PHACTR2	C8orf6, TPD52

Abbreviations: ABC, activated-B-cell-like; DLBCL, diffuse large B-cell lymphoma; MYC-R⁺, MYC rearrangement-positive; MYC-R⁻, MYC rearrangement-negative; Myc^{high}, high Myc protein expression; Myc^{low}, low Myc protein expression.

Table 5 Gene expression profile signatures of MYC-R⁺ GCB-DLBCL and MYC-R⁺ DLBCL overall

Functional categories	Upregulated	Downregulated
1. All (<i>Myc</i> ^{high} or <i>Myc</i> ^{low}) MYC-R ⁺ GCB vs all (<i>Myc</i> ^{high} or <i>Myc</i> ^{low}) MYC-R ⁻ GCB (false discovery rate < 0.01, fold change > 2.3)		
Signaling		TRAF1, DUSP4
Cell proliferation and growth, gene expression	MYC, SMAD1, STRBP, BACH2	LMO2, FAM129A, STAT3
Apoptosis	ZNF385B, PEG10	FAS, BCL2A1, TMEM49
Immune response, anti-viral/ anti-microbial activities	IGJ, DKFZp686O24166/NCR3LG1	CD58, LYZ, CHI3L1
Cell adhesion, extracellular matrix, migration		BGN, CD44
MicroRNA, long noncoding RNA		MIR155HG, MIR21, NCRNA00152/LINC00152
Unknown function		LOC283027
2. All (GCB or ABC) MYC-R ⁺ vs all (GCB or ABC) MYC-R ⁻ (false discovery rate < 0.01, fold change > 1.66)		
Signaling	BMP3	TNFAIP3
Cell proliferation and growth, gene expression	MYC, BACH2, STRBP, SMAD1	
Apoptosis	PEG10, ZNF385B	BCL2A1, CFLAR, TMEM49
Metabolism	CYP39A1, PLA2G12A	
Immune response	DKFZp686O24166/NCR3LG1	
Cell adhesion, extracellular matrix, migration	PCDH9	CD44
MicroRNA, long noncoding RNA	GAS5	MIR155HG, NCRNA00152/ LINC00152, MIR21
Transport	SLC25A27, SLC44A1, SLC35E3	
Degradation		RFFL
Unknown function	C4orf34	LOC283027
3. MYC-R ⁺ /Myc ^{high} DLBCL (GCB or ABC) vs MYC-R ⁻ /Myc ^{high} DLBCL (GCB or ABC) (false discovery rate < 0.01, fold change > 2.05)		
Signaling	BMP3, BMP7	
Transcription, ribosome biogenesis	MYC, IKZF2, SMAD1, STRBP	STAT3, BATF
Apoptosis		TMEM49, CFLAR
Immune response	DKFZp686O24166/NCR3LG1	
Cell adhesion, extracellular matrix, migration	TAPT1, PCDH9	EMILIN2, TPM4, ARHGAP25, CD44
MicroRNA, long noncoding RNA		MIR21, LINC00152/NCRNA00152
Transport	SLC35E3	
Unknown function		LOC100288765, LOC283027

Abbreviations: ABC, activated B-cell-like; DLBCL, diffuse large B-cell lymphoma; GCB, germinal center B-cell-like; MYC-R⁺, MYC rearrangement-positive; MYC-R⁻, MYC rearrangement-negative; Myc^{high}, high Myc protein expression; Myc^{low}, low Myc protein expression.

with Bcl-2 expression and MYC-R⁺ GCB-DLBCL without Bcl-2 expression. Correspondingly, patients with MYC/BCL2 double-hit lymphoma and those with single MYC rearrangements did not have significant differentially expressed genes, which is consistent with an earlier study.¹⁶ The survival of MYC-R⁺ Myc^{high}/Bcl-2^{high} GCB-DLBCL patients was markedly poorer compared with that of MYC-R⁻ Myc^{high}/Bcl-2^{high} GCB-DLBCL patients but this difference was not significant (Figures 6e and f). However, this difference was significant when we used cutoffs of $\geq 40\%$ or $> 50\%$ for Myc overexpression.

Although concurrent evaluation of Myc and Bcl-2 expression improves the specificity of Myc biomarker in DLBCL, MYC rearrangement continues to demonstrate clinical value. In our cohort, there was no significant difference in survival between patients with the GCB or ABC subtypes of MYC-R⁻ Myc^{high}/Bcl-2^{high} (ie, double-positive lymphoma). Among overall double-positive lymphoma patients, patients with MYC-R⁺ GCB double-positive lymphoma patients had significantly poorer survival compared with those double-positive lymphoma patients without MYC rearrangement (Figures 6g and h). The

survival of MYC-R⁺ double-positive lymphoma patients with the GCB or ABC subtypes appeared to be different, although *P*-values were not significant and the case numbers were small.

Discussion

Previously, we reported MYC translocation and Myc overexpression as adverse prognostic biomarkers individually.^{15,24} In this study, we analyzed the occurrence of MYC translocation and Myc overexpression in GCB- and ABC-DLBCL (Figures 2a and b), and compared with the clinical features and tumor biology associated with these two overlapping biomarkers which have not been done by previous studies, and examined the dependence/independence between their indicated prognoses. To reduce the difference in Myc expression levels as a causing factor for the differential prognostic effect and tumor biology between MYC translocation and Myc overexpression activated by other mechanisms, the cutoff for Myc^{high} was set at $\geq 70\%$ in this study, which is the optimal cutoff for predicting MYC translocation according to previous studies.^{6,7,31} Using this cutoff, the frequency

Figure 6 Prognostic analysis in Myc^{high} germinal-center-B-cell-like (GCB) diffuse large B-cell lymphoma (DLBCL) and in Myc^{high}Bcl-2^{high} DLBCL patients. (**a** and **b**) Among MYC-non-rearranged (MYC-R⁻) Myc^{high} GCB-DLBCL patients, those with Bcl-2 overexpression had significantly worse overall survival and progression-free survival compared with those who did not have Bcl-2 overexpression. (**c** and **d**) Among patients with MYC-R⁺/Myc^{high} GCB-DLBCL, those with Bcl-2 overexpression had significantly poorer progression-free survival compared with those who did not have Bcl-2 overexpression. The P-value for overall survival was not significant. (**e** and **f**) Among patients with Myc^{high}Bcl-2^{high} GCB-DLBCL, those with MYC rearrangement had poorer overall and progression-free survival compared with those without MYC rearrangement did, but these differences were not statistically significant. (**g** and **h**) Among Myc^{high}Bcl-2^{high} DLBCL patients, GCB-DLBCL patients with MYC rearrangement had significantly poorer overall and progression-free survival than Myc^{high}Bcl-2^{high} patients without MYC rearrangement did. ABC, activated-B-cell-like; Bcl-2^{high}, high Bcl-2 protein expression; Bcl-2^{low}, low Bcl-2 protein expression; GCB, germinal-center-B-cell-like; Myc^{high}, high Myc protein expression; MYC-R⁺, MYC rearrangement-positive; MYC-R⁻, MYC rearrangement-negative.

of Myc overexpression was 32.7% in overall DLBCL patients (close to the frequencies by other independent studies^{13,22,23}), 73% in MYC-R⁺ GCB-DLBCL, and 54% in MYC-R⁺ ABC-DLBCL (lower than the 93% in Green *et al*⁶ and 85% in Horn *et al*¹⁰). Tables 6–7 summarize the results of published MYC studies including ours. The current study shows that

MYC translocation and Myc overexpression in DLBCL only partially overlaps and evaluation of both is critical for stratifying patients and predicting treatment outcomes. MYC-rearranged DLBCL without Myc protein overexpression did not show significantly worse survival (Figure 1g). However, evaluation of Myc overexpression alone is also insufficient for

predicting poorer prognosis in *MYC*-R⁻ cases, especially in *MYC*-R⁻ GCB-DLBCL (Figure 5a). This low specificity for *Myc*^{high} as an adverse prognostic factor can be improved by concurrent evaluation of Bcl-2 expression (Supplementary Figures S5 and S6), which is overexpressed mainly in ABC-DLBCL and associated with *Myc* overexpression. However, the survival of *MYC*-rearranged *Myc*^{high}Bcl-2^{high} GCB double-positive lymphoma patients remains significantly worse than other double-positive lymphoma patients (Figures 6g and h).

The biological investigation (in this regard, $\geq 70\%$ is a better cutoff compared with $\geq 40\%$ for *Myc*^{high} in our cohort) revealed that *MYC* activation was associated with significantly increased or decreased expression of genes and proteins involved in cell proliferation (e.g., pAKT, Ki-67), apoptosis (p53, Bcl-2, *FAS*, *BCL2A1*, *PEG10*, *HINT1*, *TRAF1*), differentiation (*PRDM1*, BLIMP-1, *BACH2* (which represses *PRDM1*)⁴³), noncoding RNAs (eg, *LINC00152*, *GAS5*, *SNHG1*, *NAPSB*) and microRNAs, microenvironment, and immune responses, as well as cell-of-origin markers (Figure 3 and Tables 3–5). Corresponding to the differences in prognostic effect between various *Myc*^{high} subtypes, only *MYC*-R⁺/*Myc*^{high} GCB-DLBCL and *MYC*-R⁻/*Myc*^{high} ABC-DLBCL, but not *MYC*-R⁻/*Myc*^{high} GCB-DLBCL or *MYC*-R⁺/*Myc*^{high} ABC-DLBCL, demonstrated distinct gene expression profiling feature compared with the *Myc*^{low} subgroup (Figures 5b and e and Supplementary Figures S4D and F). Remarkably, *MYC*-R⁺/*Myc*^{high} GCB-DLBCL had a characteristic gene expression profiling in DLBCL. *Myc* activation was associated with gene expression profiling signatures suggesting decreased immune responses and a number of microRNAs overlapped with the molecular Burkitt lymphoma signature,^{29,30} including *MIR17HG*, which was markedly upregulated, and *MIR21* (ref. 44) and *MIR155HG*, which were significantly downregulated. Other studies have shown these microRNAs to be regulated by the *Myc*, Bcl-6, STAT3, and NF- κ B pathways, and the *MIR17HG* locus was frequently amplified in Burkitt lymphoma.^{45–48} Downregulation of *MIR155HG* expression may contribute to the pathogenesis of *MYC* translocation, as miR-155 suppresses activation-induced cytidine deaminase, which mediates *MYC/IGH* translocation.⁴⁹ These microRNA signatures may also be implicated in defining the gene expression profiling features of *MYC*-R⁺/*Myc*^{high} GCB-DLBCL. In lymphoma cells, ectopic expression of miR-155 is associated with downregulation of *IGJ*, *FAS*, *SMAD3/5*, and *BACH1*, as well as HLA genes.⁴⁵ *IGJ*, *FAS*, and genes involved in BMP/SMAD pathways (such as *SMAD1*, *BMP3*, and *BMP7*), as well as *SMARCA4* (miR-21 target gene⁵⁰), were upregulated in *MYC*-R⁺ GCB-DLBCL (Tables 3 and 5). miR-155 target gene *BCL2* showed increased Bcl-2 protein expression in ABC-DLBCL (Figure 3c). Phosphorylated SMAD proteins have roles in BMP-induced cell growth inhibition (this inhibition can be overcome via *MYC*

translocations),^{51,52} immunoregulation,⁵³ and ionizing radiation-induced double-strand break signaling.⁵⁴ In addition, in *MYC*-R⁺/*Myc*^{high} GCB-DLBCL, antiapoptotic *PEG10* and *ZNF385B*, which modulates p53 activity resulting in cell-cycle arrest over apoptosis, were significantly upregulated.

One possible reason for the differential prognoses and gene expression profiling signatures among GCB or ABC subtype of *Myc*^{high} and *MYC*-R⁺ cases is the difference in *Myc* protein levels. For example, *MYC*-R⁺/*Myc*^{high} GCB-DLBCL had highest *Myc* expression levels (Figure 2c) and significant or nonsignificant trends towards worse prognosis compared with all other three *Myc*^{high} groups. *MYC*-R⁺ cases with low *Myc* protein expression had good prognosis even though having an *MYC* rearrangement. Different *MYC* translocation partners, and/or breakpoints outside of *MYC* gene leaving *MYC* repressor element intact during rearrangement,^{4,17} may cause the low *MYC*-mRNAs in these *MYC*-R⁺/*Myc*^{low} DLBCL cases (Figures 1e–g). Compared with *MYC*-R⁺/*Myc*^{high} GCB-DLBCL, ABC subtype of *MYC*-R⁺/*Myc*^{high} DLBCL had similar *MYC*-mRNA but significantly lower *Myc* protein levels (Figure 2d) and trends of better survival. *MYC*-R⁻/*Myc*^{high} GCB-DLBCL compared with *Myc*^{low} GCB-DLBCL had significantly higher levels of *MYC*-mRNA and *Myc* protein, but the false discovery rate for *MYC* upregulation in gene expression profiling analysis was high (>0.45), which may suggest either *Myc*^{high} or *Myc*^{low} group are heterogeneous and the molecular mechanisms inducing *Myc* in this *Myc*^{high} group include post-transcriptional regulation. A previous *Myc* study in immature and mature GCB cells during germinal cell formation also demonstrated the lack of correlation between *Myc* protein and mRNA levels.⁵⁵ The lack of distinct gene expression profiling signatures and better survival of *MYC*-R⁻/*Myc*^{high} GCB-DLBCL and *MYC*-R⁺/*Myc*^{high} ABC-DLBCL compared with *MYC*-R⁺/*Myc*^{high} GCB-DLBCL may indicate lower *Myc* activities corresponding to intracellular *Myc* protein levels; however, small case numbers and/or heterogeneity among these two *Myc*^{high} DLBCL groups could also be possible causes.

However, the *Myc* immunohistochemistry levels were similar between *MYC*-R⁻/*Myc*^{high} GCB-DLBCL and *MYC*-R⁻/*Myc*^{high} ABC-DLBCL (although GCB type had slightly lower level of *MYC*-mRNA) but their prognosis showed differences. *MYC*-R⁺/*Myc*^{high} ABC-DLBCL had similar *Myc* protein level to that of *MYC*-R⁻/*Myc*^{high} ABC-DLBCL but only the latter showed typical *Myc* gene expression profiling signatures (Figure 2c). Therefore, *Myc*-associated molecular mechanisms in GCB or ABC subtype of *MYC*-R⁺/*Myc*^{high} cases impacted the prognostic and biological effect of *Myc*. GCB and ABC subtypes of *Myc*^{high} DLBCL had difference in frequencies of *TP53* mutation, *MYC* translocation, *BCL2* translocation, and Bcl-2 expression, as well as cell-of-origin biomarkers (Table 1 and Figure 3). *MYC*-R⁺/*Myc*^{high}

Table 6 Summary of frequencies and prognostic significance of *MYC*-R in DLBCL in the literature and reported by the current study

References	DLBCL cohort	MYC-R ⁺ frequency	Significant prognostic value
van Imhoff <i>et al.</i> ¹⁸	N= 59	15%	Nonsignificant trends toward inferior survival
Savage <i>et al.</i> ¹²	N= 137	8.8%	Poorer OS and PFS
Oberman <i>et al.</i> ¹⁴	N= 220	4%	Poorer survival
Tibiletti <i>et al.</i> ¹⁹	N= 74	15.8%	No prognostic significance
Barrans <i>et al.</i> ¹¹	N= 245	14%	Poorer OS
Tapia <i>et al.</i> ⁷	N= 45	20%	
Akyurek <i>et al.</i> ⁵⁶	N= 239	6%	Poorer OS and trend of poorer PFS (<i>P</i> =0.09) in GCB
Kluk <i>et al.</i> ⁵	N= 56	9%	
Green <i>et al.</i> ⁶	N= 219	15%	
		65% of Myc ^{high} cases	
Green <i>et al.</i> ²⁵	N= 189	11%	Poorer OS
Johnson <i>et al.</i> ²²	N= 290	11.7%	Inferior OS and PFS when concurrent with Bcl-2 ^{high}
Horn <i>et al.</i> ¹³	N= 407	8.8%	Poorer EFS and OS
Valera <i>et al.</i> ²⁶	N= 176	7%	Poorer OS and PFS
Aukema <i>et al.</i> ¹⁶	N= 863	19.5%	
Tzankov <i>et al.</i> ¹⁵	N= 432	9%	Poorer disease-specific survival
Wang <i>et al.</i> ⁸	N= 135	24%	
Horn <i>et al.</i> ¹⁰	N= 111	18%	
Horn <i>et al.</i> ²⁷	N= 103	14%	Nonsignificant trend towards poorer OS (<i>P</i> =0.082)
Current study	N= 344 DLBCL	11.6%	Poorer PFS
	N= 166 GCB	16.3%	Poorer OS and PFS
	N= 177 ABC	7.3%	No prognostic significance
	N= 107 Myc ^{high} cases	24.3%	Poorer PFS
	N= 175*		
	N= 233 Myc ^{low} cases	5.6%	No prognostic significance
	N= 360*		(a nonsignificant trend of better OS: <i>P</i> =0.25)
	N= 42 Myc ^{high} GCB	45.2%	Poorer OS and PFS
	N= 76*		
	N= 65 Myc ^{high} ABC	10.8%	No prognostic significance
	N= 98*		
	N= 60 Myc ^{high} /Bcl-2 ^{high} DPL	23.3%	GCB subtype of MYC-R ⁺ Myc ^{high} /Bcl-2 ^{high} DPL (16.7%) had significantly poorer OS and PFS among Myc ^{high} /Bcl-2 ^{high} DPL
	N= 98*		
		Occurrence of GCB vs ABC subtype of MYC-R ⁺ : 2.1; Occurrence of GCB vs ABC subtype of MYC-R ⁺ /Myc ^{high} : 2.7; Occurrence of GCB vs ABC subtype of MYC-R ⁺ /Myc ^{high} /Bcl-2 ^{high} : 2.5 Prognosis of GCB vs ABC subtype of MYC-R ⁺ : trends towards poorer OS and PFS (<i>P</i> =0.11); Prognosis of GCB vs ABC subtype of MYC-R ⁺ /Myc ^{high} : no significant difference (slightly unfavorable trends); Prognosis of GCB vs ABC subtype of MYC-R ⁺ /Myc ^{high} /Bcl-2 ^{high} : unfavorable trends (for PFS, <i>P</i> =0.098)	

Abbreviations: ABC, activated-B-cell like; Bcl-2^{high}, high Bcl-2 protein expression; DLBCL, diffuse large B-cell lymphoma; DPL, double-positive lymphoma; EFS, event-free survival; GCB, germinal center B-cell like; Myc^{high}, high Myc protein expression; Myc^{low}, low Myc protein expression; MYC-R⁺, MYC rearrangement positive; MYC-R⁻, MYC rearrangement negative; OS, overall survival; PFS, progression-free survival.

Note: Case numbers marked by * are the total Myc^{high} or Myc^{low} case numbers (with or without MYC-R status determined).

and MYC-R⁻/Myc^{high} DLBCL had significantly different levels of MDM2, the cell-of-origin markers, and CD30 (in GCB-DLBCL) and pAKT (in ABC-DLBCL) (Figure 4), as well as significantly different gene expression profiling signatures at the mRNA level (Figure 5i). Recently, studies have posited a 'c-Myc function rule,' in which c-Myc is a 'universal amplifier' of active (expressed) genes in lymphocytes.^{33,34,57}

In summary, both MYC rearrangement and Myc overexpression have advantage and limitations as a single biomarker in DLBCL, and their prognostic importance is significantly different in GCB- vs ABC-DLBCL. GCB subtype (opposite to the general association of ABC-DLBCL with poorer survival)

of MYC-R⁺ DLBCL with Myc overexpression was associated with significantly poorer survival, likely contributed by significantly higher Myc protein levels as well as associated tumor biology. In ABC-DLBCL, Myc overexpression associated with Bcl-2 overexpression was a significantly adverse biomarker independent of MYC translocation. Our results suggest that fluorescence *in situ* hybridization analysis for MYC rearrangements and immunohistochemistry evaluation for Myc and Bcl-2 expression are both needed to determine the prognosis in subsets of patients.⁵⁷ Insights gained into the tumor biology associated with MYC abnormalities are important for understanding the functional role of MYC in lymphomagenesis and chemoresistance,

Table 7 Summary of frequencies and prognostic significance of Myc overexpression (Myc^{high}) and MYC mRNA levels in DLBCL in the literature and reported by the current study*Myc overexpression*

References	DLBCL cohort	Cutoff for Myc ^{high} (%)	Myc ^{high} frequency	Significant prognostic value
Kluk <i>et al.</i> ⁵	N=77	> 50	19.5%	Poorer OS
Green <i>et al.</i> ⁶	N=205	≥ 70	17%	
Green <i>et al.</i> ²⁵	N=193	≥ 40		Poorer OS and PFS when concurrent with Bcl-2 ^{high}
Johnson <i>et al.</i> ²²	N=307	≥ 40	33%	Inferior OS and PFS when concurrent with Bcl-2 ^{high}
Hu <i>et al.</i> ²⁴	N=466	≥ 40	64%	Concurrent Myc ^{high} /Bcl-2 ^{high} correlated with poorer OS and PFS
Horn <i>et al.</i> ¹³	N=283	> 40	31.8%	Poorer OS and PFS
Valera <i>et al.</i> ²⁶	N=168	10	48%	Inferior OS and PFS
		40	13%	
Perry <i>et al.</i> ²³	N=106	> 50	35%	Poorer OS and EFS
Horn <i>et al.</i> ¹⁰	N=39	≥ 80	77–85% of MYC-R ⁺ cases; 19–46% of MYC-R ⁻ cases	
Horn <i>et al.</i> ²⁷	N=92	≥ 30	49%	Nonsignificant trends toward poorer OS (<i>P</i> =0.08) and poorer PFS (<i>P</i> =0.091)
Current study	N=535	≥ 70	32.7%	Poorer OS and PFS
	N=272	≥ 70	27.9% in GCB	Poorer OS and PFS
	N=259	≥ 70	37.8% in ABC	Poorer OS and PFS
	N=40	≥ 70	67% of MYC-R ⁺ cases	Poorer OS and PFS
	N=304	≥ 70	26.9% of MYC-R ⁻ cases	Poorer OS and PFS
	N=26	≥ 70	73% of MYC-R ⁺ GCB	Trends toward poorer OS (<i>P</i> =0.07) and PFS
	N=136	≥ 70	16.9% of MYC-R ⁻ GCB	Nonsignificant trends toward poorer OS (<i>P</i> =0.40) and poorer PFS (<i>P</i> =0.48)
	N=13	≥ 70	54% of MYC-R ⁺ ABC	Trends toward poorer OS and PFS (<i>P</i> =0.07)
	N=164	≥ 70	35.4% of MYC-R ⁻ ABC	Poorer OS and PFS

Occurrence of ABC vs GCB subtype of Myc^{high}: 1.3 (in the overall DLBCL cohort, case numbers of ABC vs GCB subtype: 0.95);
Occurrence of ABC vs GCB subtype of Myc^{high}/Bcl-2^{high}: 1.8
Percentage of MYC-R⁻/Myc^{high} ABC-DLBCL among all Myc^{high}: 54.2%;
Percentage of MYC-R⁺/Myc^{high} GCB-DLBCL among all Myc^{high}: 17.8%
Prognosis of ABC vs GCB subtype of Myc^{high}: no significant difference (slightly unfavorable trends);
Prognosis of ABC vs GCB subtype of Myc^{high}/Bcl-2^{high}: no significant difference;
MYC-R⁻/Myc^{high} ABC vs MYC-R⁻/Myc^{high} GCB: trend of poorer OS (for both OS and PFS: *P*=0.14);
MYC-R⁺/Myc^{high} ABC-DLBCL vs MYC-R⁻/Myc^{high} GCB-DLBCL: nonsignificantly poorer OS (*P*=0.35) and PFS (*P*=0.25);
MYC-R⁺/Myc^{high} GCB-DLBCL vs MYC-R⁻/Myc^{high} ABC-DLBCL: a trend towards poorer PFS (*P*=0.058)

MYC mRNA levels (3 groups: low, intermediate, and high MYC-mRNA)

References	Frequency of MYC-mRNA ^{high}	Significant prognostic value
Current study	N=471	Poorer OS and PFS
	N=241	Poorer OS and PFS
	N=228	Poorer OS and a trend towards poorer PFS (<i>P</i> =0.069)
	N=33	Poorer OS and a trend towards poorer PFS (<i>P</i> =0.066)
	N=265	Trend towards poorer OS (<i>P</i> =0.06)
	Prognosis of ABC vs GCB subtype of MYC-mRNA ^{high} : no significant difference (slightly unfavorable trends)	

Abbreviations: ABC, activated B-cell like; Bcl-2^{high}, high Bcl-2 protein expression; DLBCL, diffuse large B-cell lymphoma; EFS, event-free survival; GCB, germinal center B-cell like; Myc^{high}, high Myc protein expression; Myc^{low}, low Myc protein expression; MYC-R⁻, MYC rearrangement-negative; MYC-R⁺, MYC rearrangement positive; OS, overall survival; PFS, progression-free survival.

and help identify oncogenic targets for therapeutic intervention (Table 7).

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Author contributions

Conception and design: ZYX-M, KHY; research performance: ZYX-M, KHY; provision of study materials, key reagents and technology: ZYX-M, BSD, XW, MT, GCM, AT, YX, LZ, CV, KD, LY, AC, AO, YZ, GB, KLR, EDH, WWLC, JHvK, MP, AJMF, MBM, BMP, XZ, JNW, MAP, TJM, RNM, YL, LJM, KHY; collection and assembly of data under approved IRB and MTA: ZYX-M, BSD, XW, AT, YX, CV, KD, AC, AO, YZ, GB, KLR, EDH, WWLC, JHvK, MP, AJMF, MBM, BMP, XZ, JNW, MAP, KHY; data analysis and interpretation: ZYX-M, KHY; manuscript writing: ZYX-M, LJM, KHY; final approval of manuscript: all authors.

Disclosure/conflict of interest

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