

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

Xu-Monette, Zijun Y; Dabaja, Bouthaina S; Wang, Xiaoxiao; Tu, Meifeng; Manyam, Ganiraju C; Tzankov, Alexander; Xia, Yi; Zhang, Li Li; Sun, Ruifang; Visco, Carlo; Dybkaer, Karen; Yin, Lihui; Chiu, April; Orazi, Attilio; Zu, Youli; Bhagat, Govind; Richards, Kristy L; Hsi, Eric D; Choi, William W L; van Krieken, J Han; Huh, Jooryung; Ponzoni, Maurilio; Ferreri, Andrés J M; Møller, Michael Boe; Parsons, Ben M; Zhao, Xiaoying; Winter, Jane N; Piris, Miguel A; McDonnell, Timothy J; Miranda, Roberto N; Li, Yong; Medeiros, L Jeffrey; Young, Ken H

*Published in:*
Modern Pathology

*DOI:*
10.1038/modpathol.2015.118

*Publication date:*
2015

*Document version*
Publisher's PDF, also known as Version of record

*Document license*
CC BY-NC-ND

*Citation for pulished version (APA):*
Xu-Monette, Z. Y., Dabaja, B. S., Wang, X., Tu, M., Manyam, G. C., Tzankov, A., ... Young, K. H. (2015). Clinical features, tumor biology, and prognosis associated with MYC rearrangement and Myc overexpression in diffuse large B-cell lymphoma patients treated with rituximab-CHOP. Modern Pathology, 28(12), 1555-73. DOI: 10.1038/modpathol.2015.118

Modern Pathology (2015) 28, 1555–1573



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

Zijun Y Xu-Monette[1], Bouthaina S Dabaja[2], Xiaoxiao Wang[1], Meifeng Tu[1], Ganiraju C Manyam[3], Alexander Tzankov[4], Yi Xia[1], Li Zhang[3], Ruifang Sun[1], Carlo Visco[5], Karen Dybkaer[6], Lihui Yin[1], April Chiu[7], Attilio Orazi[8], Youli Zu[9], Govind Bhagat[10], Kristy L Richards[11], Eric D Hsi[12], William WL Choi[13], J Han van Krieken[14], Jooryung Huh[15], Maurilio Ponzoni[16], Andrés JM Ferreri[16], Michael B Møller[17], Ben M Parsons[18], Xiaoying Zhao[19], Jane N Winter[20], Miguel A Piris[21], Timothy J McDonnell[1], Roberto N Miranda[1], Yong Li[22], L Jeffrey Medeiros[1] and Ken H Young[1,23]

*[1]Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA; [2]Department of Radiation Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA; [3]Department of Bioinformatics and Computational Biology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA; [4]University Hospital, Basel, Switzerland; [5]San Bortolo Hospital, Vicenza, Italy; [6]Aalborg University Hospital, Aalborg, Denmark; [7]Memorial Sloan-Kettering Cancer Center, New York, NY, USA; [8]Weill Medical College of Cornell University, New York, NY, USA; [9]The Methodist Hospital, Houston, TX, USA; [10]Columbia University Medical Center and New York Presbyterian Hospital, New York, NY, USA; [11]University of North Carolina School of Medicine, Chapel Hill, NC, USA; [12]Cleveland Clinic, Cleveland, OH, USA; [13]University of Hong Kong Li Ka Shing Faculty of Medicine, Hong Kong, China; [14]Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; [15]Asan Medical Center, Ulsan University College of Medicine, Seoul, South Korea; [16]San Raffaele H Scientific Institute, Milan, Italy; [17]Odense University Hospital, Odense, Denmark; [18]Gundersen Lutheran Health System, La Crosse, WI, USA; [19]Zhejiang University School of Medicine, Second University Hospital, Hangzhou, China; [20]Feinberg School of Medicine, Northwestern University, Chicago, IL, USA; [21]Hospital Universitario Marqués de Valdecilla, Santander, Spain; [22]Lerner Research Institute, Cleveland Clinic, Cleveland, OH, USA and [23]The University of Texas School of Medicine, Graduate School of Biomedical Sciences, Houston, TX, USA*

**_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**

Correspondence: Dr KH Young, MD, Department of Hematopathology, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030, USA.
E-mail: khyoung@mdanderson.org
Received 10 July 2015; revised 16 August 2015; accepted 29 August 2015; published online 6 November 2015

**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.**
*Modern Pathology* (2015) **28,** 1555–1573; doi:10.1038/modpathol.2015.118; published online 6 November 2015

*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 genes[2] 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-$\kappa$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 $\beta$-uniform mixture method.

### Statistical Analysis

The clinical and pathobiological features of DLBCL patients at the time of presentation were compared using the $\chi^2$ 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

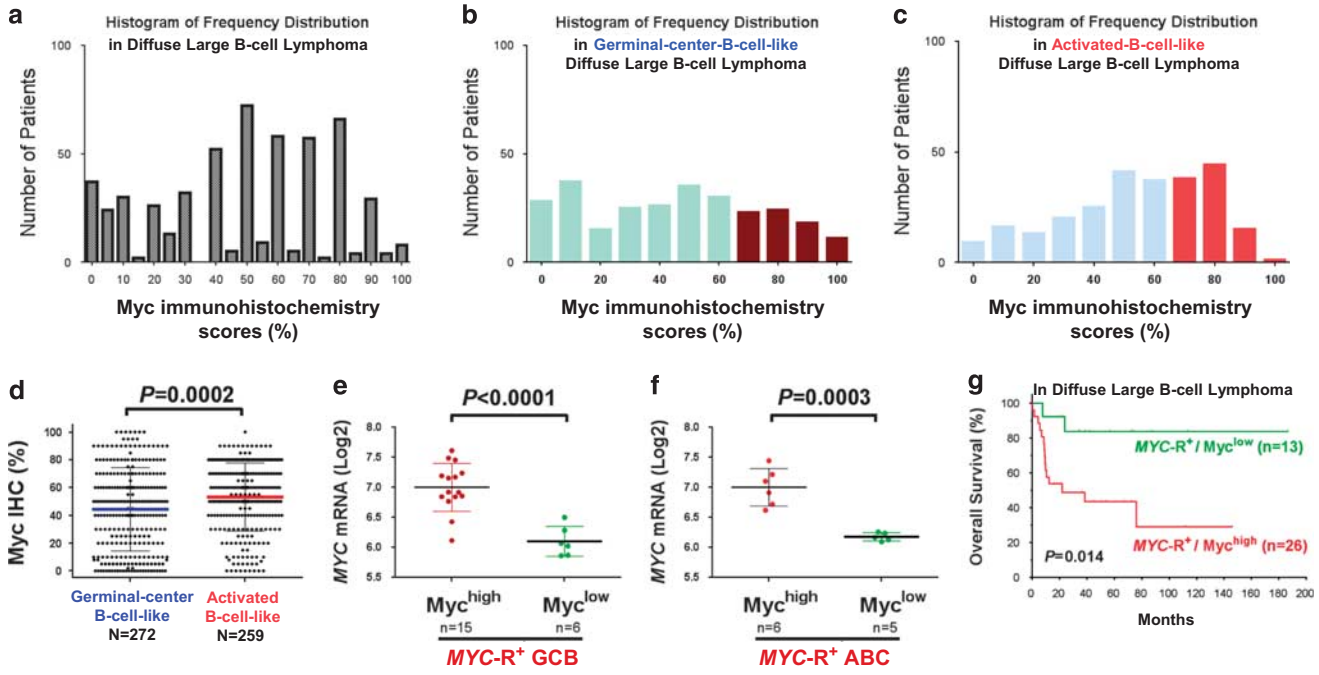


**Figure 1** (**a**–**c**) Histograms of Myc expression according to immunohistochemistry staining in all diffuse large B-cell lymphoma (DLBCL) patients, germinal center B-cell-like (GCB) DLBCL patients, and activated B-cell-like (ABC) DLBCL patients. (**d**) The mean Myc protein levels of ABC-DLBCL 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, $\geq 2$ extranodal sites, ECOG performance status $\geq 2$, tumor size $\geq 5$ 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 $\geq 60$ 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).

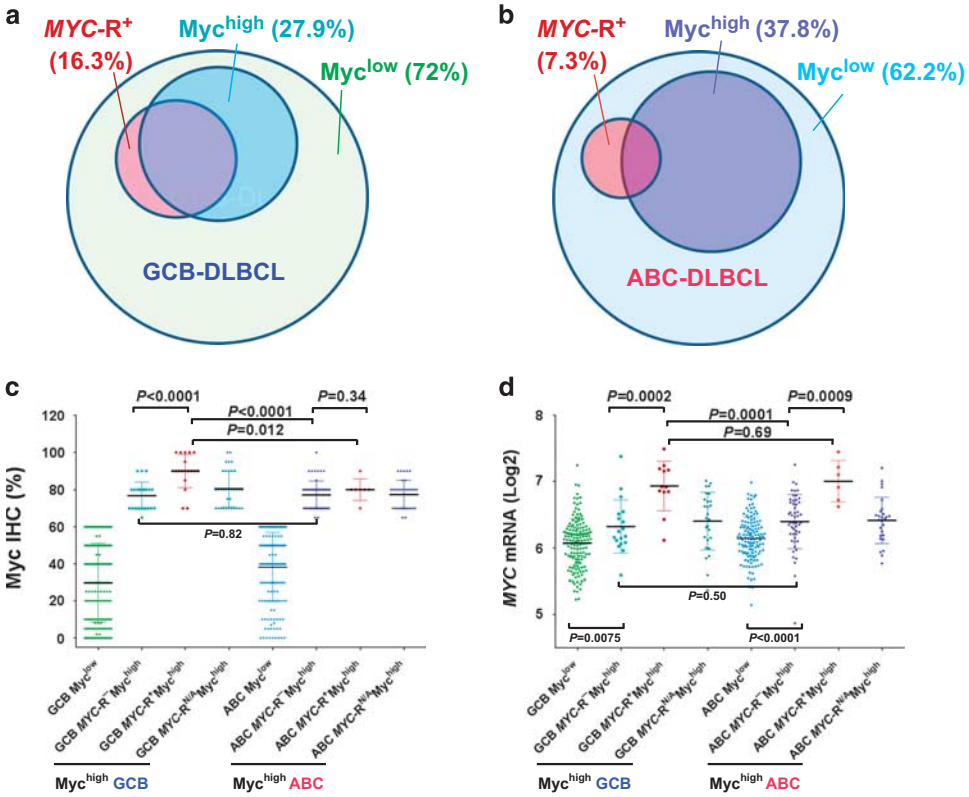


**Figure 2** Occurrence of *MYC* rearrangement (*MYC*-R) and overexpression (Myc$^{high}$) and *MYC* expression levels in diffuse large B-cell lymphoma (DLBCL). (**a** and **b**) Schematic diagrams showing the frequencies of *MYC* translocation (*MYC*-R$^+$) and Myc overexpression (Myc$^{high}$) and 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-$\kappa$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]

| | *DLBCL* | | $P_1$ *values* | *GCB-DLBCL* | | $P_2$ *values* | *ABC-DLBCL* | | $P_3$ *values* | $P_4$ *values* |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Myc*$^{high}$ N *(%)* | *Myc*$^{low}$ N *(%)* | | *Myc*$^{high}$ N *(%)* | *Myc*$^{low}$ N *(%)* | | *Myc*$^{high}$ N *(%)* | *Myc*$^{low}$ N *(%)* | | |
| Variables | 175 (100) | 360 (100) | | 76 (100) | 196 (100) | | 98 (100) | 161 (100) | | **0.016** |
| *Age (years)* | | | | | | | | | | |
| <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) | | |
| *Gender* | | | | | | | | | | |
| 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) | | |
| *Stage* | | | | | | | | | | |
| 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) | | |
| *B-symptoms* | | | | | | | | | | |
| 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) | | |
| *LDH level* | | | | | | | | | | |
| 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) | | |
| *Number of extranodal sites* | | | | | | | | | | |
| 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) | | |
| *ECOG performance status score* | | | | | | | | | | |
| 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) | | |
| *Size of largest tumor (cm)* | | | | | | | | | | |
| <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) | | |
| *IPI score* | | | | | | | | | | |
| 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) | | |
| *Therapy response* | | | | | | | | | | |
| 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 | | |
| *Primary origin* | | | | | | | | | | |
| 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) | | |
| *Bone marrow involvement* | | | | | | | | | | |
| 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) | | |
| *Ki-67 index (%)* | | | | | | | | | | |
| <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) | | |
| TP53 *mutations* | | | | | | | | | | |
| 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) | | |
| MYC *translocation* | | | | | | | | | | |
| 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) | | |
| BCL2 *translocation* | | | | | | | | | | |
| 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) | | |
| BCL6 *translocation* | | | | | | | | | | |
| 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) | | |
| *Bcl-2 expression* | | | | | | | | | | |
| <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.
[a]With 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

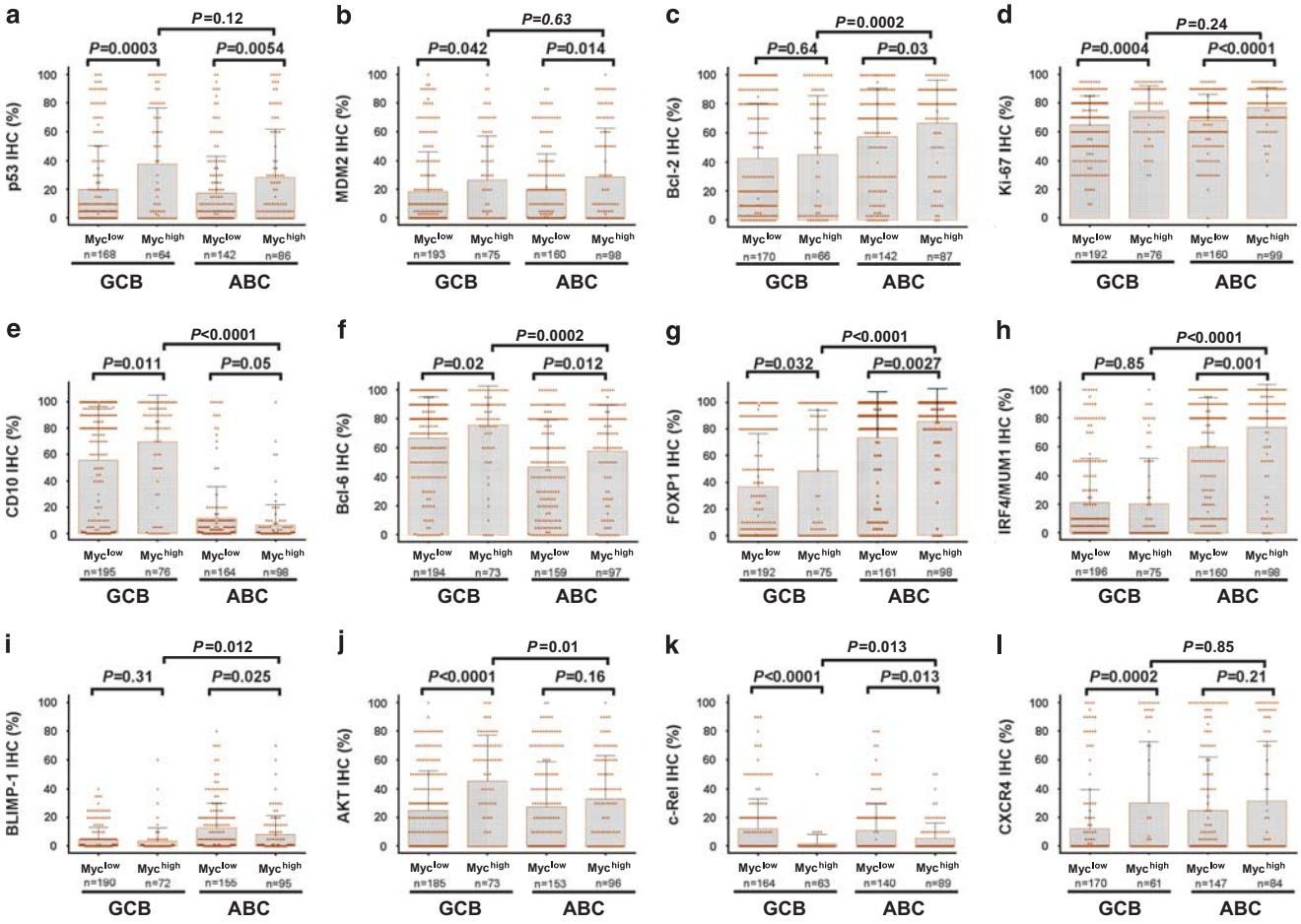


**Figure 3** Expression of pathobiological markers in germinal center B-cell-like (GCB) and activated B-cell-like (ABC) diffuse large B-cell lymphoma with or without Myc overexpression. In both GCB- and ABC-DLBCL, Myc overexpression was associated with significantly higher levels of p53 (**a**), MDM2 (**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).

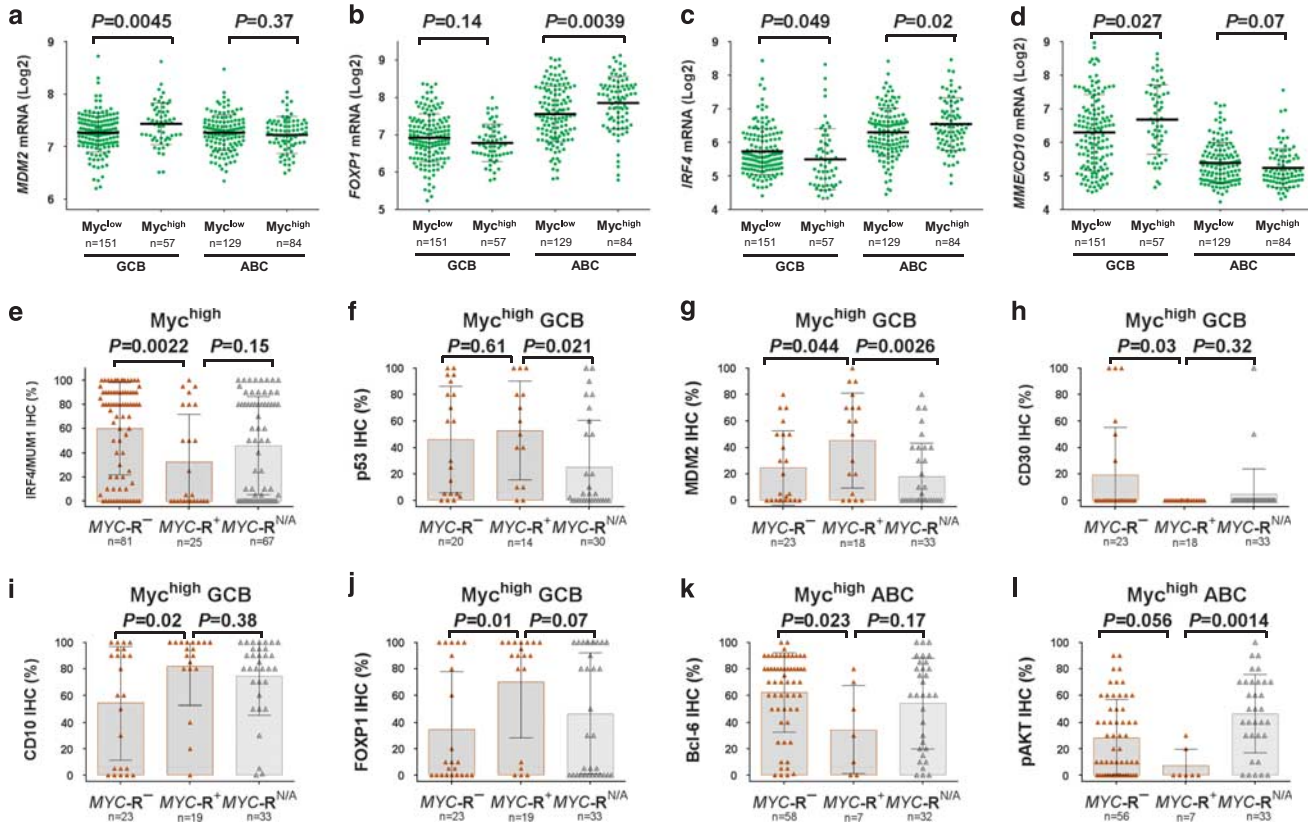


**Figure 4** (**a–d**) Myc overexpression was correlated with significantly higher levels of *MDM2*, *IRF4*, and *MME*/*CD10* mRNA expression in germinal center B-cell-like (GCB) diffuse large B-cell lymphoma and significantly higher levels of *FOXP1* and *IRF4* mRNA expression in activated B-cell-like (ABC) diffuse large B-cell lymphoma (DLBCL). (**e**) The *MYC*-R$^+$/Myc$^{high}$ compared with *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* |
|---|---|---|---|
| | *Myc$^{high}$ vs Myc$^{low}$ (false discovery rate < 0.05, fold change > 1.53)* | *Myc$^{high}$ vs Myc$^{low}$ (false discovery rate < 0.01, fold change > 1.68)* | *Myc$^{high}$ vs Myc$^{low}$ (false discovery rate < 0.01, fold change > 1.31)* |
| Upregulated | *MYC*, *AICDA*, *SNHG1*, *SNHG4*, *TMEM97*, *PAICS*, *TCL1A*, *XK*, *FAM129C*, *CKS2*, *PEG10*, *IGF2BP3*, *SLC16A1*, *FAM72A/B/C/D*, *HELLS*, *CDCA7L*, *MAD2L1*, *MRPL3*, *PRO2964*, *C13orf18*, *MIR17HG* | *MYC*, *PEG10*, *SNHG4*, *STRBP*, *CYP39A1*, *DKFZp686O24166*, *PAICS*, *FAM72A/B/C/D*, *CDC25A*, *SLC16A1*, *RPS21*, *DEPDC1*, *HSPD1* | *MYC*, *MAD2L1*, *EEF1E1*, *RUVBL2*, *SNHG1*, *RG9MTD1*, *MRPL3*, *IPO7*, *CCDC86*, *TFAM*, *GAR1*, *MATR3*, *SNHG4*, *TOMM5*, *NOC3L*, *WDR43*, *DDX21*, *LYAR*, *RPL24*, *LOC388796*, *SNHG8*, *WDR75*, *DCTPP1*, *MAT2A*, *QDPR*, *APEX1* |
| Downregulated | *CD3E*, *HOPX*, *TRBC1*, *COL3A1*, *RGS1*, *COL3A1*, *TRBC1*, *ITM2A*, *GZMK*, *ITGB5*, *ITM2A* | *MIR155HG*, *TRBC1*, *GABBR1/UBD*, *CD58*, *CD3E*, *BHLHE41*, *GZMK*, *DUSP4*, *TRBC1*, *SLAMF7*, *LCP2*, *RGS1*, *ITM2A*, *SKI*, *SLAMF8*, *CD44*, *LOC285628*, *FYB*, *CCL5*, *MDFIC*, *CCND2*, *BCL11B*, *TNFAIP3*, *SLFN5*, *SNX9*, *IL10RA*, *GBP2* | |

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).

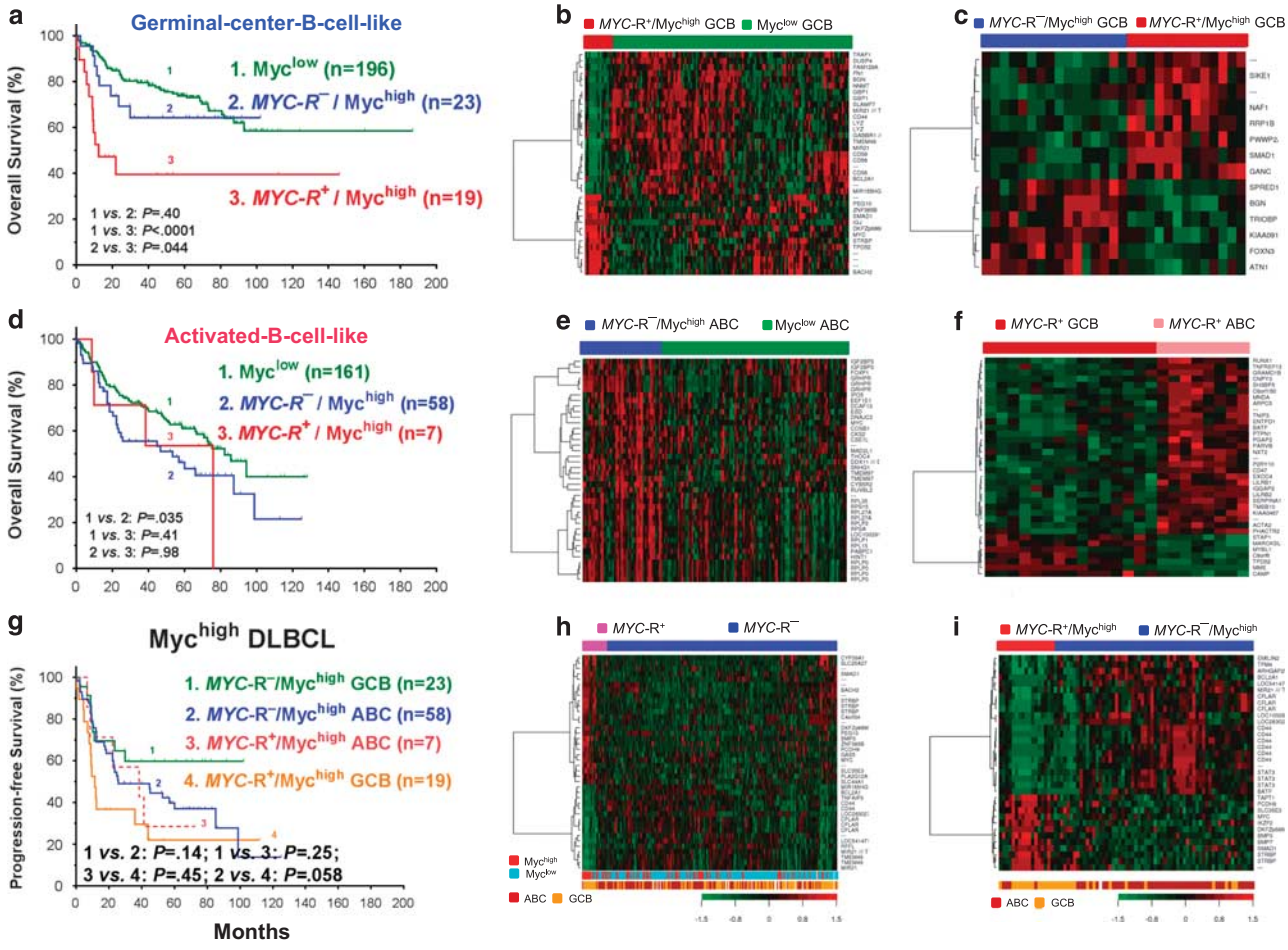


**Figure 5** Combined prognostic and biologic analysis of germinal-center-B-cell-like (GCB) and activated-B-cell-like (ABC) diffuse large B-cell lymphoma (DLBCL) with or without *MYC* translocation (*MYC*-R) and/or Myc overexpression (Myc$^{high}$) in the current study cohort. (**a**) GCB-DLBCL patients 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*, *MNDA*, *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 (Myc$^{high}$ or Myc$^{low}$)* MYC-*R$^+$ GCB vs all (Myc$^{high}$ or Myc$^{low}$)* 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

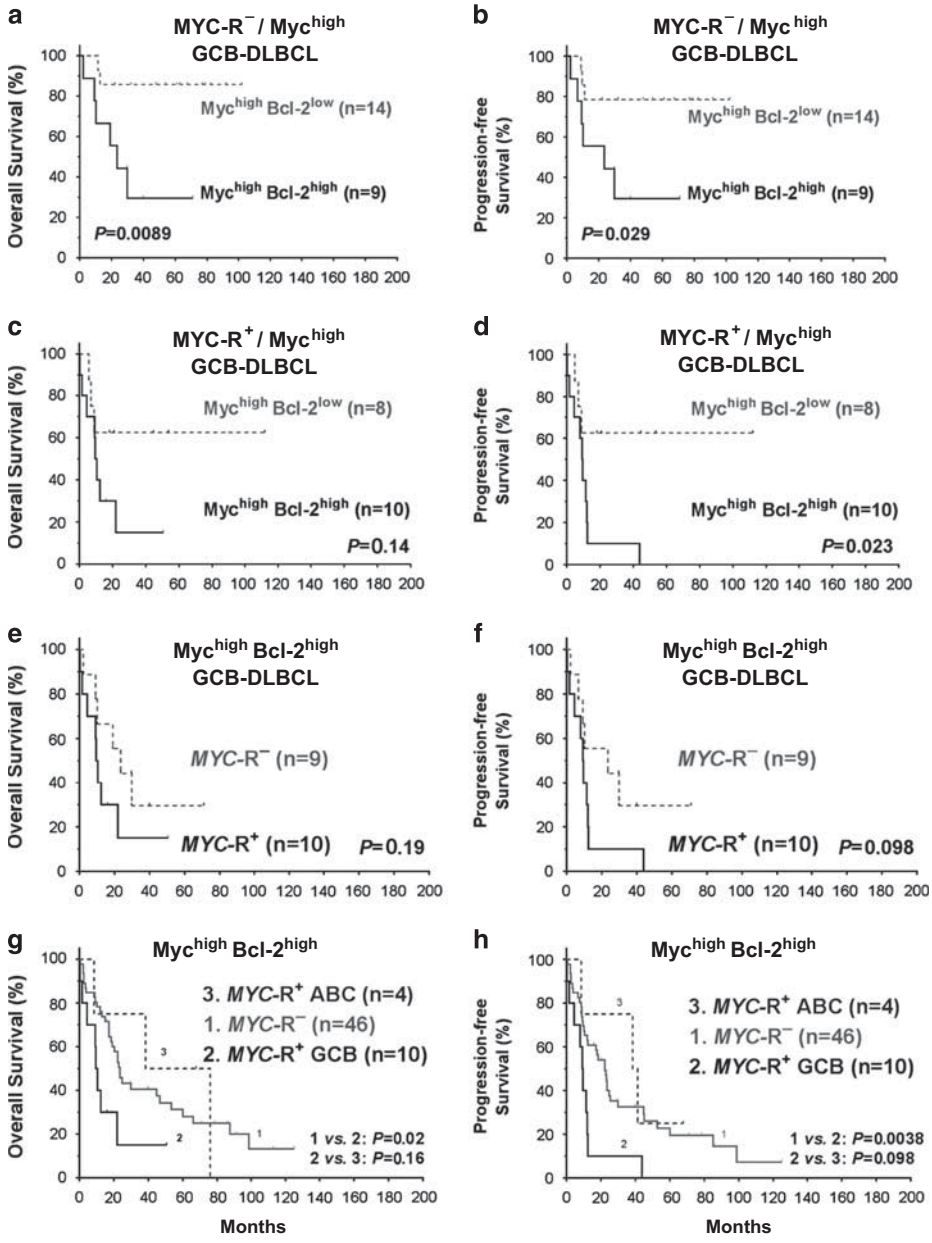


**Figure 6** Prognostic analysis in Myc$^{high}$ germinal-center-B-cell-like (GCB) diffuse large B-cell lymphoma (DLBCL) and in Myc$^{high}$Bcl-2$^{high}$ DLBCL patients. (**a** and **b**) Among *MYC*-non-rearranged (*MYC*-R$^{-}$) Myc$^{high}$ GCB-DLBCL patients, those with Bcl-2 overexpression had 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-$\kappa$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 gene[50]), 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 overcame 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 *et al.*[18] | $N=59$ | | 15% | Nonsignificant trends toward inferior survival |
| Savage *et al.*[12] | $N=137$ | | 8.8% | Poorer OS and PFS |
| Oberman *et al.*[14] | $N=220$ | | 4% | Poorer survival |
| Tibiletti *et al.*[19] | $N=74$ | | 15.8% | No prognostic significance |
| Barrans *et al.*[11] | $N=245$ | | 14% | Poorer OS |
| Tapia *et al.*[7] | $N=45$ | | 20% | |
| Akyurek *et al.*[56] | $N=239$ | | 6% | Poorer OS and trend of poorer PFS ($P=0.09$) in GCB |
| Kluk *et al.*[5] | $N=56$ | | 9% | |
| Green *et al.*[6] | $N=219$ | | 15% | |
| | | | 65% of Myc$^{high}$ cases | |
| Green *et al.*[25] | $N=189$ | | 11% | Poorer OS |
| Johnson *et al.*[22] | $N=290$ | | 11.7% | Inferior OS and PFS when concurrent with Bcl-2$^{high}$ |
| Horn *et al.*[13] | $N=407$ | | 8.8% | Poorer EFS and OS |
| Valera *et al.*[26] | $N=176$ | | 7% | Poorer OS and PFS |
| Aukema *et al.*[16] | $N=863$ | | 19.5% | |
| Tzankov *et al.*[15] | $N=432$ | | 9% | Poorer disease-specific survival |
| Wang *et al.*[8] | $N=135$ | | 24% | |
| Horn *et al.*[10] | $N=111$ | | 18% | |
| Horn *et al.*[27] | $N=103$ | | 14% | Nonsignificant trend towards poorer OS ($P=0.082$) |
| 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: $P=0.25$) |
| | $N=42$ | 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 ($P=0.11$); | |
| | | | 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, $P=0.098$) | |

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-DCLBL) (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 *et al*.[5] | $N=77$ | >50 | 19.5% | Poorer OS |
| Green *et al*.[6] | $N=205$ | ≥70 | 17% | |
| Green *et al*.[25] | $N=193$ | ≥40 | | Poorer OS and PFS when concurrent with $Bcl\text{-}2^{high}$ |
| Johnson *et al*.[22] | $N=307$ | ≥40 | 33% | Inferior OS and PFS when concurrent with $Bcl\text{-}2^{high}$ |
| Hu *et al*.[24] | $N=466$ | ≥40 | 64% | Concurrent $Myc^{high}/Bcl\text{-}2^{high}$ correlated with poorer OS and PFS |
| Horn *et al*.[13] | $N=283$ | >40 | 31.8% | Poorer OS and PFS |
| Valera *et al*.[26] | $N=168$ | 10 | 48% | Inferior OS and PFS |
| | | 40 | 13% | |
| Perry *et al*.[23] | $N=106$ | >50 | 35% | Poorer OS and EFS |
| Horn *et al*.[10] | $N=39$ | ≥80 | 77–85% of *MYC*-$R^+$ cases; 19–46% of *MYC*-$R^-$ cases | |
| Horn *et al*.[27] | $N=92$ | ≥30 | 49% | Nonsignificant trends toward poorer OS ($P=0.08$) and poorer PFS ($P=0.091$) |
| 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 ($P=0.07$) and PFS |
| | $N=136$ | ≥70 | 16.9% of *MYC*-$R^-$ GCB | Nonsignificant trends toward poorer OS ($P=0.40$) and poorer PFS ($P=0.48$) |
| | $N=13$ | ≥70 | 54% of *MYC*-$R^+$ ABC | Trends toward poorer OS and PFS ($P=0.07$) |
| | $N=164$ | ≥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 $Myc^{high}/Bcl\text{-}2^{high}$: 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 $Myc^{high}/Bcl\text{-}2^{high}$: 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 | $N=471$ | 15.7% of DLBCL | Poorer OS and PFS |
| | $N=241$ | 16.6% in GCB | Poorer OS and PFS |
| | $N=228$ | 14.9% in ABC | Poorer OS and a trend towards poorer PFS ($P=0.069$) |
| | $N=33$ | 55% of *MYC*-$R^+$ cases | Poorer OS and a trend towards poorer PFS ($P=0.066$) |
| | $N=265$ | 12.1% of *MYC*-$R^-$ cases | Trend towards poorer OS ($P=0.06$) |
| | | Prognosis of ABC *vs* GCB subtype of *MYC*-$mRNA^{high}$: no significant difference (slightly unfavorable trends) | |

Abbreviations: ABC, activated B-cell like; $Bcl\text{-}2^{high}$, 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).

## Acknowledgments

This study was supported by the National Cancer Institute/National Institutes of Health (R01CA138688, R01CA187415 and 1RC1CA146299 to YL and KHY). ZYXM is the recipient of the Harold C and Mary L Daily Endowment Fellowships and Shannon Timmins Fellowship for Leukemia Research Award. GM is supported by a grant from the Michael and Susan Dell Foundation. KHY is supported by The University of Texas MD Anderson

Cancer Center Lymphoma Moonshot Program, Institutional Research and Development Fund, an Institutional Research Grant Award, an MD Anderson Cancer Center Lymphoma Specialized Programs on Research Excellence (SPORE) Research Development Program Award, an MD Anderson Cancer Center Myeloma SPORE Research Development Program Award, a Gundersen Lutheran Medical Foundation Award, and partially supported by the National Cancer Institute/National Institutes of Health (P50CA136411 and P50CA142509), and by MD Anderson's Cancer Center Support Grant CA016672.

## 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

KHY also receives research support from Roche Molecular System, Gilead Sciences Pharmaceutical, Seattle Genetics, Dai Sanyo Pharmaceutical, Adaptive Biotechnology, and HTG Molecular Diagnostics.

## References

1 Felsher DW. MYC inactivation elicits oncogene addiction through both tumor cell-intrinsic and host-dependent mechanisms. Genes Cancer 2010;1:597–604.
2 Delmore JE, Issa GC, Lemieux ME *et al.* BET bromodomain inhibition as a therapeutic strategy to target c-Myc. Cell 2011;146:904–917.
3 Chesi M, Matthews GM, Garbitt VM *et al.* Drug response in a genetically engineered mouse model of multiple myeloma is predictive of clinical efficacy. Blood 2012;120:376–385.
4 Ott G, Rosenwald A, Campo E. Understanding MYC-driven aggressive B-cell lymphomas: pathogenesis and classification. Blood 2013;122:3884–3891.
5 Kluk MJ, Chapuy B, Sinha P *et al.* Immunohistochemical detection of MYC-driven diffuse large B-cell lymphomas. PLoS One 2012;7:e33813.
6 Green TM, Nielsen O, de Stricker K *et al.* High levels of nuclear MYC protein predict the presence of MYC rearrangement in diffuse large B-cell lymphoma. Am J Surg Pathol 2012;36:612–619.
7 Tapia G, Lopez R, Munoz-Marmol AM *et al.* Immunohistochemical detection of MYC protein correlates with MYC gene status in aggressive B cell lymphomas. Histopathology 2011;59:672–678.
8 Wang XJ, Medeiros LJ, Lin P *et al.* MYC cytogenetic status correlates with expression and has prognostic significance in patients with MYC/BCL2 protein double-positive diffuse large B-cell lymphoma. Am J Surg Pathol 2015;39:1250–1258.
9 Cheah CY, Oki Y, Westin JR *et al.* A clinician's guide to double hit lymphomas. Br J Haematol 2015;168:784–795.
10 Horn H, Staiger AM, Vohringer M *et al.* Diffuse large B-cell lymphomas of immunoblastic type are a major reservoir for MYC-IGH translocations. Am J Surg Pathol 2015;39:61–66.
11 Barrans S, Crouch S, Smith A *et al.* Rearrangement of MYC is associated with poor prognosis in patients with diffuse large B-cell lymphoma treated in the era of rituximab. J Clin Oncol 2010;28:3360–3365.
12 Savage KJ, Johnson NA, Ben-Neriah S *et al.* MYC gene rearrangements are associated with a poor prognosis in diffuse large B-cell lymphoma patients treated with R-CHOP chemotherapy. Blood 2009;114:3533–3537.
13 Horn H, Ziepert M, Becher C *et al.* MYC status in concert with BCL2 and BCL6 expression predicts outcome in diffuse large B-cell lymphoma. Blood 2013;121:2253–2263.
14 Obermann EC, Csato M, Dirnhofer S *et al.* Aberrations of the MYC gene in unselected cases of diffuse large B-cell lymphoma are rare and unpredictable by morphological or immunohistochemical assessment. J Clin Pathol 2009;62:754–756.
15 Tzankov A, Xu-Monette ZY, Gerhard M *et al.* Rearrangements of MYC gene facilitate risk stratification in diffuse large B-cell lymphoma patients treated with rituximab-CHOP. Mod Pathol 2014;27:958–971.
16 Aukema SM, Kreuz M, Kohler CW *et al.* Biological characterization of adult MYC-translocation-positive mature B-cell lymphomas other than molecular Burkitt lymphoma. Haematologica 2014;99:726–735.
17 Bertrand P, Bastard C, Maingonnat C *et al.* Mapping of MYC breakpoints in 8q24 rearrangements involving non-immunoglobulin partners in B-cell lymphomas. Leukemia 2007;21:515–523.
18 van Imhoff GW, Boerma EJ, van der Holt B *et al.* Prognostic impact of germinal center-associated proteins and chromosomal breakpoints in poor-risk diffuse large B-cell lymphoma. J Clin Oncol 2006;24:4135–4142.
19 Tibiletti MG, Martin V, Bernasconi B *et al.* BCL2, BCL6, MYC, MALT 1, and BCL10 rearrangements in nodal diffuse large B-cell lymphomas: a multicenter evaluation of a new set of fluorescent *in situ* hybridization probes and correlation with clinical outcome. Hum Pathol 2009;40:645–652.
20 Johnson NA, Savage KJ, Ludkovski O *et al.* Lymphomas with concurrent BCL2 and MYC translocations: the critical factors associated with survival. Blood 2009;114:2273–2279.
21 Visco C, Tzankov A, Xu-Monette ZY *et al.* Patients with diffuse large B-cell lymphoma of germinal center origin with BCL2 translocations have poor outcome, irrespective of MYC status: a report from an International DLBCL rituximab-CHOP Consortium Program Study. Haematologica 2013;98:255–263.
22 Johnson NA, Slack GW, Savage KJ *et al.* Concurrent expression of MYC and BCL2 in diffuse large B-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. J Clin Oncol 2012;30:3452–3459.

23 Perry AM, Alvarado-Bernal Y, Laurini JA *et al.* MYC and BCL2 protein expression predicts survival in patients with diffuse large B-cell lymphoma treated with rituximab. Br J Haematol 2014;165:382–391.
24 Hu S, Xu-Monette ZY, Tzankov A *et al.* MYC/BCL2 protein coexpression contributes to the inferior survival of activated B-cell subtype of diffuse large B-cell lymphoma and demonstrates high-risk gene expression signatures: a report from The International DLBCL Rituximab-CHOP Consortium Program. Blood 2013;121:4021–4031.
25 Green TM, Young KH, Visco C *et al.* Immunohistochemical double-hit score is a strong predictor of outcome in patients with diffuse large B-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. J Clin Oncol 2012;30:3460–3467.
26 Valera A, Lopez-Guillermo A, Cardesa-Salzmann T *et al.* MYC protein expression and genetic alterations have prognostic impact in patients with diffuse large B-cell lymphoma treated with immunochemotherapy. Haematologica 2013;98:1554–1562.
27 Horn H, Ziepert M, Wartenberg M *et al.* Different biological risk factors in young poor-prognosis and elderly patients with diffuse large B-cell lymphoma. Leukemia 2015;29:1564–1570.
28 Pfreundschuh M. Growing importance of MYC/BCL2 immunohistochemistry in diffuse large B-cell lymphomas. J Clin Oncol 2012;30:3433–3435.
29 Hummel M, Bentink S, Berger H *et al.* A biologic definition of Burkitt's lymphoma from transcriptional and genomic profiling. N Engl J Med 2006;354: 2419–2430.
30 Dave SS, Fu K, Wright GW *et al.* Molecular diagnosis of Burkitt's lymphoma. N Engl J Med 2006;354:2431–2442.
31 Thieblemont C, Briere J. MYC, BCL2, BCL6 in DLBCL: impact for clinics in the future? Blood 2013;121:2165–2166.
32 Albihn A, Johnsen JI, Henriksson MA. MYC in oncogenesis and as a target for cancer therapies. Adv Cancer Res 2010;107:163–224.
33 Lin CY, Loven J, Rahl PB *et al.* Transcriptional amplification in tumor cells with elevated c-Myc. Cell 2012;151:56–67.
34 Nie Z, Hu G, Wei G *et al.* c-Myc is a universal amplifier of expressed genes in lymphocytes and embryonic stem cells. Cell 2012;151:68–79.
35 Visco C, Li Y, Xu-Monette ZY *et al.* Comprehensive gene expression profiling and immunohistochemical studies support application of immunophenotypic algorithm for molecular subtype classification in diffuse large B-cell lymphoma: a report from the International DLBCL Rituximab-CHOP Consortium Program Study. Leukemia 2012;26:2103–2113.
36 Xu-Monette ZY, Wu L, Visco C *et al.* Mutational profile and prognostic significance of TP53 in diffuse large B-cell lymphoma patients treated with R-CHOP: report from an International DLBCL Rituximab-CHOP Consortium Program Study. Blood 2012;120:3986–3996.
37 Xu-Monette ZY, Moller MB, Tzankov A *et al.* MDM2 phenotypic and genotypic profiling, respective to TP53 genetic status, in diffuse large B-cell lymphoma patients treated with rituximab-CHOP immunochemotherapy: a report from the International DLBCL Rituximab-CHOP Consortium Program. Blood 2013;122:2630–2640.
38 Hu S, Xu-Monette ZY, Balasubramanyam A *et al.* CD30 expression defines a novel subgroup of diffuse large B-cell lymphoma with favorable prognosis and distinct gene expression signature: a report from the International DLBCL Rituximab-CHOP Consortium Program Study. Blood 2013;121:2715–2724.
39 Ok CY, Xu-Monette ZY, Li L *et al.* Evaluation of NF-kappaB subunit expression and signaling pathway activation demonstrates that p52 expression confers better outcome in germinal center B-cell-like diffuse large B-cell lymphoma in association with CD30 and BCL2 functions. Mod Pathol 2015;28:1202–1213.
40 Chen J, Xu-Monette ZY, Deng L *et al.* Dysregulated CXCR4 expression promotes lymphoma cell survival and independently predicts disease progression in germinal center B-cell-like diffuse large B-cell lymphoma. Oncotarget 2015;6:5597–5614.
41 Li L, Xu-Monette ZY, Ok CY *et al.* Prognostic Impact of c-Rel nuclear expression and REL amplification and crosstalk between c-Rel and the p53 pathway in diffuse large B-cell lymphoma. Oncotarget; advance online publication, 30 June 2015 [e-pub ahead of print].
42 Liu Z, Xu-Monette ZY, Cao X *et al.* Prognostic and biological significance of survivin expression in patients with diffuse large B-cell lymphoma treated with rituximab-CHOP therapy. Mod Pathol; advance online publication, 7 August 2015 [e-pub ahead of print].
43 Huang C, Geng H, Boss I *et al.* Cooperative transcriptional repression by BCL6 and BACH2 in germinal center B-cell differentiation. Blood 2014;123: 1012–1020.
44 Lu TX, Hartner J, Lim EJ *et al.* MicroRNA-21 limits *in vivo* immune response-mediated activation of the IL-12/IFN-gamma pathway, Th1 polarization, and the severity of delayed-type hypersensitivity. J Immunol 2011;187:3362–3373.
45 Iqbal J, Shen Y, Huang X *et al.* Global microRNA expression profiling uncovers molecular markers for classification and prognosis in aggressive B-cell lymphoma. Blood 2015;125:1137–1145.
46 Di Lisio L, Sanchez-Beato M, Gomez-Lopez G *et al.* MicroRNA signatures in B-cell lymphomas. Blood Cancer J 2012;2:e57.
47 Basso K, Schneider C, Shen Q *et al.* BCL6 positively regulates AID and germinal center gene expression via repression of miR-155. J Exp Med 2012;209: 2455–2465.
48 Testoni M, Zucca E, Young KH *et al.* Genetic lesions in diffuse large B-cell lymphomas. Ann Oncol 2015; 1069–1080.
49 Dorsett Y, McBride KM, Jankovic M *et al.* MicroRNA-155 suppresses activation-induced cytidine deaminase-mediated Myc-Igh translocation. Immunity 2008;28:630–638.
50 Schramedei K, Morbt N, Pfeifer G *et al.* MicroRNA-21 targets tumor suppressor genes ANP32A and SMARCA4. Oncogene 2011;30:2975–2985.
51 Holien T, Vatsveen TK, Hella H *et al.* Bone morphogenetic proteins induce apoptosis in multiple myeloma cells by Smad-dependent repression of MYC. Leukemia 2012;26:1073–1080.
52 Huse K, Bakkebo M, Walchli S *et al.* Role of Smad proteins in resistance to BMP-induced growth inhibition in B-cell lymphoma. PLoS One 2012;7:e46117.
53 Malhotra N, Kang J. SMAD regulatory networks construct a balanced immune system. Immunology 2013;139:1–10.
54 Wang M, Saha J, Hada M *et al.* Novel Smad proteins localize to IR-induced double-strand breaks: interplay

between TGFbeta and ATM pathways. Nucleic Acids Res 2013;41:933–942.
55 Calado DP, Sasaki Y, Godinho SA *et al.* The cell-cycle regulator c-Myc is essential for the formation and maintenance of germinal centers. Nat Immunol 2012;13:1092–1100.
56 Akyurek N, Uner A, Benekli M *et al.* Prognostic significance of MYC, BCL2, and BCL6 rearrangements in patients with diffuse large B-cell lymphoma treated with cyclophosphamide, doxorubicin, vincristine, and prednisone plus rituximab. Cancer 2012;118: 4173–4183.
57 Cai Q, Medeiros LJ, Young KH. MYC-driven aggressive B-cell lymphomas: biology, entity, differential diagnosis and clinical management. Oncotarget 2015 [e-pub ahead of print].

Supplementary Information accompanies the paper on Modern Pathology website (http://www.nature.com/modpathol)