Check for updates

Clinical impact of ibrutinib plus R-CHOP in untreated DLBCL coexpressing BCL2 and MYC in the phase 3 PHOENIX trial

Peter W. M. Johnson,[1] Sriram Balasubramanian,[2,*] Brendan Hodkinson,[3] S. Martin Shreeve,[4] Steven Sun,[3] Srimathi Srinivasan,[5] Andrew J. Steele,[3,†] Jessica Vermeulen,[6] Laurie H. Sehn,[7] and Wyndham H. Wilson[8]

[1]Centre for Cancer Immunology, University of Southampton, Southampton General Hospital, Southampton, United Kingdom; [2]Oncology Translational Research, Janssen Research & Development, San Diego, CA; [3]Oncology Translational Research, Janssen Research & Development, Spring House, PA; [4]Clinical Oncology, Janssen Research & Development, San Diego, CA; [5]Oncology Translational Research, Janssen Research & Development, Lower Gwynedd Township, PA; [6]Clinical Oncology, Janssen Research & Development, Leiden, The Netherlands; [7]British Columbia Cancer Centre for Lymphoid Cancer, University of British Columbia, Vancouver, BC, Canada; and [8]Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD

Key Points

- In the PHOENIX trial, the addition of ibrutinib to R-CHOP did not improve the survival of patients with previously untreated non-GCB DLBCL.

- This study identified a patient subset with high *BCL2/MYC* coexpression using RNA sequencing, with improved EFS after R-CHOP with ibrutinib.

Diffuse large B-cell lymphoma (DLBCL), with high coexpression of BCL2 and MYC proteins (DE lymphoma), is considered an adverse prognostic indicator associated mostly with non-germinal center B-cell–like (non-GCB) DLBCL. BCL2/MYC overexpression is associated with B-cell receptor (BCR) pathway activation; consequently, DE DLBCL may be sensitive to BCR inhibitors. We assessed whether high *BCL2/MYC* coexpression by RNA sequencing could identify a patient subset responsive to ibrutinib using baseline biopsies from the PHOENIX trial, which evaluated the addition of ibrutinib to rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) in untreated non-GCB DLBCL. *BCL2/MYC* RNA expression was correlated with lower event-free survival (EFS) and overall survival (OS) using Kaplan–Meier estimates with Cox regression and log-rank testing. In total, 234 of 766 (30.5%) patients had high *BCL2/MYC* coexpression: 123 of 386 (31.9%) received ibrutinib plus R-CHOP and 111 of 380 (29.2%) received R-CHOP. EFS was superior with ibrutinib plus R-CHOP compared with R-CHOP alone in patients with high *BCL2/MYC* coexpression, but there was no significant impact on OS. However, EFS and OS showed clinically meaningful improvement with ibrutinib plus R-CHOP over R-CHOP alone in patients aged <60 years with high *BCL2/MYC* coexpression. We observed a significant association between high *BCL2/MYC* coexpression and activated B-cell-like and *MYD88^{L265P}/CD79B*-mutated subtypes of DLBCL. Consequently, high *BCL2/MYC* coexpression identified a subset of non-GCB DLBCL that may be preferentially responsive to ibrutinib and warrants further investigation. This trial was registered at www.clinicaltrials.gov as #NCT01855750.

Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma (NHL), representing 25% to 30% of all NHL cases worldwide.[1] It is recognized that DLBCL is a heterogenous disease characterized by distinct, pathologic subtypes and gene expression profiles impacting clinical outcomes.[2,3] Studies have shown that rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) chemoimmunotherapy, the standard frontline treatment for DLBCL,[4] is effective in approximately 60% of patients,[5] emphasizing the need for alternative therapeutic options.

Gene expression profiling (GEP) identified 2 molecular DLBCL subtypes according to the differentiation stage of the B-cell the cancer originated from: germinal center B-cell–like (GCB) and activated B-cell–like (ABC), together comprising 80% to 85% of cases, with the remaining cases considered unclassified.[6,7] Using the Hans algorithm, DLBCL can also be dichotomously classified by immunohistochemistry (IHC) into GCB and non-GCB (non-GCB includes ABC by GEP plus several unclassified cases).[8] Patients with DLBCL of ABC origin (and most non-GCB by IHC) have constitutive activation of the NF-κB pathway required for survival of this type of lymphoma,[9] and they are at higher risk of treatment failure following R-CHOP therapy.[3,7,10]

More recent molecular subclassification attempts of DLBCL tumors have proposed to further subdivide the ABC subgroup into genetic subtypes such as MCD (characterized by $MYD88^{L265P}$ and/or $CD79B$ mutations), BN2 (characterized by $BCL6$ fusions and/or $NOTCH2$ mutations), N1 (characterized by $NOTCH1$ mutations), and A53 (aneuploid with $TP53$ inactivation), among others.[11-13] These genetic subtypes have distinct pathogeneses and phenotypic properties and may respond differently to therapies,[11,13-15] which remains to be proven prospectively.

$BCL2$ and MYC genes are key regulators of cell survival[16] and proliferation,[17] respectively, and deregulation of these genes is a well-known adverse prognostic factor in patients with DLBCL treated with standard-of-care therapy. Overexpression of $BCL2$ and MYC genes can occur because of chromosomal translocations, copy number aberrations, and transcriptional/translational changes.[18-21] Based on the underlying molecular aberrations, DLBCL featuring $BCL2$ and MYC overexpression can be placed into 2 categories. Several studies using IHC have identified that a significant proportion of DLBCL cases are characterized by high coexpression of BCL2 and MYC proteins, which is associated with poor prognosis following R-CHOP treatment.[20-22] DLBCL with high coexpression of BCL2 and MYC proteins, or double-expressor (DE) lymphoma,[2] is found primarily in the non-GCB (or ABC) subtype of DLBCL.[22] These DLBCLs are genetically distinct from those with MYC and $BCL2$ chromosomal rearrangements (double-hit), which are almost all of GCB origin and are now classified by the World Health Organization as high-grade B-cell lymphoma.[2]

Ibrutinib, a first-in-class, once-daily, oral, covalent Bruton's tyrosine kinase (BTK) inhibitor, is approved in the United States for the treatment of adult patients with various B-cell malignancies, including chronic lymphocytic leukemia/small lymphocytic lymphoma, Waldenström's macroglobulinemia, previously treated mantle cell lymphoma, and previously treated marginal zone lymphoma.[23] The preferential antitumor activity of ibrutinib against the non-GCB subtype of DLBCL was demonstrated in an early phase clinical trial,[24] likely owing to the reduction in NF-κB activity through the inhibition of BTK.[25] Improved responses with ibrutinib plus R-CHOP were also reported in a small phase 1b trial in previously untreated CD20-positive NHL.[26,27]

In the phase 3 PHOENIX trial (NCT01855750) in patients with previously untreated non-GCB DLBCL, the addition of ibrutinib to R-CHOP did not improve event-free survival (EFS; primary end point) or overall survival (OS) in the intent-to-treat (ITT) and ABC populations.[28] A preplanned analysis showed a significant interaction between treatment effect and age, with patients aged <60 years deriving significant clinical benefit from ibrutinib plus R-CHOP compared with placebo plus R-CHOP (EFS: hazard ratio [HR], 0.579; 95% confidence interval [CI], 0.380-0.881; $P = .0099$; OS: HR, 0.330; 95% CI, 0.162-0.673; $P = .0013$).[28] No survival benefit was seen in patients ≥60 years, likely because of increased toxicity in the ibrutinib plus R-CHOP arm, leading to reduced R-CHOP administration and, consequently, therapeutic benefit.[28]

As MYC and BCL2 overexpression in ABC DLBCL can be correlated with the activation of B-cell receptor (BCR) and NF-κB signaling,[25,29,30] and the BCR signaling pathway may be more active in BCL2/MYC DE DLBCL,[31] targeting these pathways with BCR inhibitors might improve outcomes in patients with high BCL2/MYC coexpression. Moreover, a recent study that characterized the genetic subtypes of DLBCL in the PHOENIX trial identified MCD and N1 subtypes (2 of 4 genetic subtypes of ABC DLBCL) to be associated with clinical benefit with ibrutinib plus R-CHOP.[12] We hypothesized that high BCL2/MYC coexpression may identify a subset of patients with non-GCB DLBCL DE lymphoma in the PHOENIX trial who were more responsive to ibrutinib treatment. We performed an exploratory analysis using baseline tumor biopsies from the PHOENIX trial to evaluate the expression levels of MYC and $BCL2$ using RNA sequencing (RNA-seq) in both treatment arms. We used RNA-seq rather than IHC because of the small amount of tissue available for the analysis. Our goal was to identify patients with high $BCL2/MYC$ coexpression and to determine whether there was a correlation between the expression data and EFS and OS in the overall population and in patients aged <60 and ≥60 years, respectively.

Methods

Patients and study design

A detailed description of the PHOENIX study has been published previously; the study was approved by the institutional review board or independent ethics committee at each participating institution and conducted in accordance with ethical principles defined by the Declaration of Helsinki and the International Conference on Harmonisation Guidelines for Good Clinical Practice. All patients provided written informed consent.[28] Briefly, this randomized, placebo-controlled, phase 3 study enrolled patients with previously untreated non-GCB DLBCL (N = 838) to receive either ibrutinib (oral 560 mg/day) plus R-CHOP (intravenous rituximab 375 mg/m^2, cyclophosphamide 750 mg/m^2, doxorubicin 50 mg/m^2, vincristine 1.4 mg/m^2 [maximum total, 2 mg], and oral prednisone [or equivalent] 100 mg) for 6 or 8 21-day cycles, or placebo plus R-CHOP.[28] The primary end point was EFS in the ITT population (non-GCB per

IHC assessment) and the ABC DLBCL subgroup (ABC per GEP assessment). The secondary end points were progression-free survival (PFS), OS, and safety. An exploratory analysis evaluated the efficacy and safety by age (<60 and ≥60 years). Response was assessed by investigators using computed tomography scans, per the Revised Response Criteria for Malignant Lymphoma.[32]

Gene expression analysis by RNA-seq

Pretreatment formalin-fixed paraffin-embedded biopsy samples were collected at baseline. RNA was extracted, prepared with TruSeq RNA Access, and profiled on an Illumina NextSeq 500 instrument (Q^2 Solutions; IQVIA, Morrisville, NC) with 100bp paired-end reads and 30 to 60 million read pairs per library. Raw sequence reads were aligned to the hs37d5 genome using STAR v2.5. 1b and gene-level quantification was performed using RSEM v1.2.23.

The median transcript per million (TPM) mapped reads for *BCL2* and *MYC* gene expression across all patients with RNA-seq data (n = 766) was used as a cutoff between high and low expression for each gene. The expression level determined by RNA-seq was compared with matching BCL2 IHC data from 184 patients for whom IHC data were available. Based on high or low BCL2 expression by IHC (low expression, <50% positive lymphoma cells; high expression, ≥50% positive lymphoma cells), an RNA-seq cutoff that minimized the sum of the percentage misclassified in each group was determined. This cutoff value was the same as the median RNA-seq expression value for *BCL2* (13.3 TPM; supplemental Figure 1), validating the use of a median TPM value for the larger data set. The median TPM value of *MYC* was 9.31. The relationship between expression by RNA-seq and survival (EFS and OS) in the 2 study arms was analyzed using Cox regression to determine HRs and log-rank testing to assess significance. The association between HTG EdgeSeq (for ABC/non-ABC subtyping; HTG Molecular Diagnostics, Tucson, AZ) cell of origin calls[28,33] and DE lymphoma status was determined using Fisher's exact test. The calls for the MCD genetic subtype were derived from the published literature,[12] and the association with DE lymphoma was tested using the statistical method described above.

Mutation detection and analysis

For biopsies from the non-China cohort of the PHOENIX study, targeted sequencing was performed at an average depth of 707× on 99 genes that were recurrently mutated in patients with lymphoma (summarized in Wilson et al[12]) using the NuGEN Ovation Custom Target Enrichment System (NuGen, San Carlos, CA). Whole exome sequencing in the PHOENIX China cohort was performed using a SureSelect Human All Exon V6 capture kit and SureSelect[XT] Reagent Kit (Agilent, Santa Clara, CA) for library preparation. Sequencing was performed using the NovaSeq 6000 System (Illumina, San Diego, CA) with an average depth of 167×. Variants were called, annotated, and filtered as previously published,[34] but using Mutect2 and rejecting any variants that were multiallelic and/or did not pass standard Mutect2 filters (either "PASS" or "clustered_events" alone was required in the "filter" field). In addition, for results from the NuGEN Ovation Target Enrichment System, UMI-tools 0.3.6 was used for deduplication, and more stringent filtering criteria were applied: a minimum sequencing depth of 70 and a minimum alternate allele frequency of 0.09 were required for variants called from those samples. Gene-/variant-level mutation frequencies in patients with high

BCL2/MYC coexpression by RNA-seq were compared with no *BCL2/MYC* coexpression, using Fisher exact test to identify genes associated with high *BCL2/MYC* coexpression.

Results

Patients and treatment

Detailed baseline demographics and primary efficacy/safety results for the PHOENIX study have been reported previously.[28] Briefly, between October 2013 and November 2015, 838 patients (ITT) with untreated non-GCB DLBCL were randomly assigned to ibrutinib plus R-CHOP (n = 419) or placebo plus R-CHOP (n = 419). Of the 747 evaluable patients, 567 (75.9%) had the ABC subtype.[28] In the primary study, the median age was 62.0 years and the median time from diagnosis to treatment initiation was 27 days. More patients discontinued treatment in the ibrutinib plus R-CHOP arm than in the placebo plus R-CHOP arm (22.4% vs 13.6%), mostly because of adverse events. Median follow-up for both arms was 34.8 months.[28]

This exploratory analysis included 766 patients for whom RNA-seq data were available: 386 from the ibrutinib plus R-CHOP arm and 380 from the placebo plus R-CHOP arm. The baseline demographics and disease characteristics of the 766 patients included in this analysis (supplemental Table 1) were similar to those of the primary study population.[28] The median age was 62.0 years, 53.7% of patients were male, median time from diagnosis was 26 days, and 68.9% of patients had ≥1 extranodal disease site. The majority of patients had advanced tumor stage (Ann Arbor III-IV, 76.2%) and had an Eastern Cooperative Oncology Group performance status (ECOG PS) score of 0 to 1 (88.0%), and 43.9% were in the revised International Prognostic Index (R-IPI) high-risk group based on information in the clinical database.

BCL2 and *MYC* expression by RNA-seq

Of the 766 patients for whom RNA-seq data for *BCL2* or *MYC* expression status were available, 234 (30.5%) showed high *BCL2/MYC* coexpression (Table 1). The proportion of patients with high *BCL2/MYC* coexpression was similar between the ibrutinib plus R-CHOP (123/386 [31.9%]) and placebo plus R-CHOP (111/380 [29.2%]) treatment arms (Table 1). Similarly, the proportion of patients with high *BCL2/MYC* coexpression was comparable between younger (<60 years; 97/317 [30.6%]) and older (≥60

Table 1. Patients with high *BCL2* and/or high *MYC* expression according to treatment arm and age

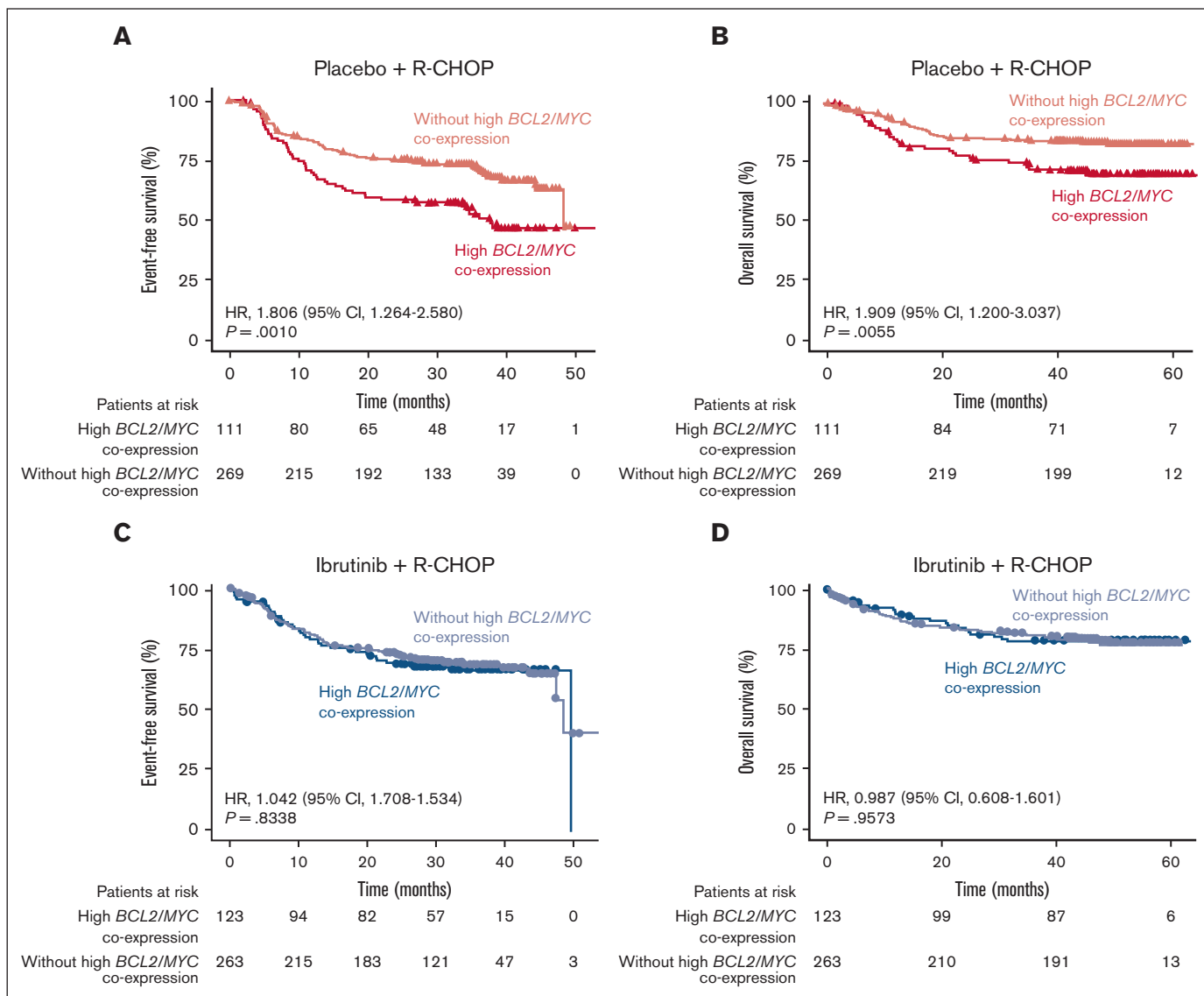| n (%)	Ibrutinib + R-CHOP (n = 386)	Placebo + R-CHOP (n = 380)
All ages		
BCL2-high	196 (50.8)	187 (49.2)
MYC-high	199 (51.6)	183 (48.2)
MYC-high + *BCL2*-high	123 (31.9)	111 (29.2)
<60 y		
n	149	168
MYC-high + *BCL2*-high	47 (31.5)	50 (29.8)
≥60 y		
n	237	212
MYC-high + *BCL2*-high	76 (32.1)	61 (28.8)

Figure 1. EFS and OS in patients with or without high *BCL2/MYC* coexpression. Overall EFS and OS in patients with or without high *BCL2/MYC* coexpression determined by RNA-seq in the placebo plus R-CHOP (A,B) and ibrutinib plus R-CHOP (C,D) arms.

years; 137/449 [30.5%]) patients (Table 1). There were no substantial differences in baseline demographic and clinical characteristics between patients with (n = 234) and without (n = 532) high *BCL2/MYC* coexpression (supplemental Table 1).

High *BCL2* and *MYC* coexpression by RNA-seq and outcomes

Response rate. Of 123 patients with high *BCL2/MYC* coexpression by RNA-seq in the ibrutinib plus R-CHOP arm, 83/123 (67.5%) achieved a complete response (CR) and 28/123 (22.8%) achieved a partial response (PR) (supplemental Table 2). In the placebo plus R-CHOP arm, of 111 patients with high *BCL2/MYC* coexpression by RNA-seq, 72/111 (64.9%) had CR and 31/111 (27.9%) had PR (supplemental Table 2).

Survival. In the placebo plus R-CHOP arm, patients with high *BCL2/MYC* coexpression (n = 111) had poorer EFS (HR, 1.806; 95% CI, 1.264-2.580; *P* = .0010; Figure 1A) and OS (HR, 1.909; 95% CI, 1.200-3.037; *P* = .0055; Figure 1B) than those without high *BCL2/MYC* coexpression (n = 269). The poor prognosis of R-CHOP–treated patients with high *BCL2/MYC* coexpression determined by this RNA-seq analysis is consistent with the results from studies evaluating coexpression of MYC and BCL2 proteins by IHC.[20-22]

In contrast, in the ibrutinib plus R-CHOP arm, there was no difference in either EFS or OS between patients with high *BCL2/MYC* coexpression (n = 123) and those without (n = 263), suggesting a potential benefit from ibrutinib treatment (Figure 1C-D).
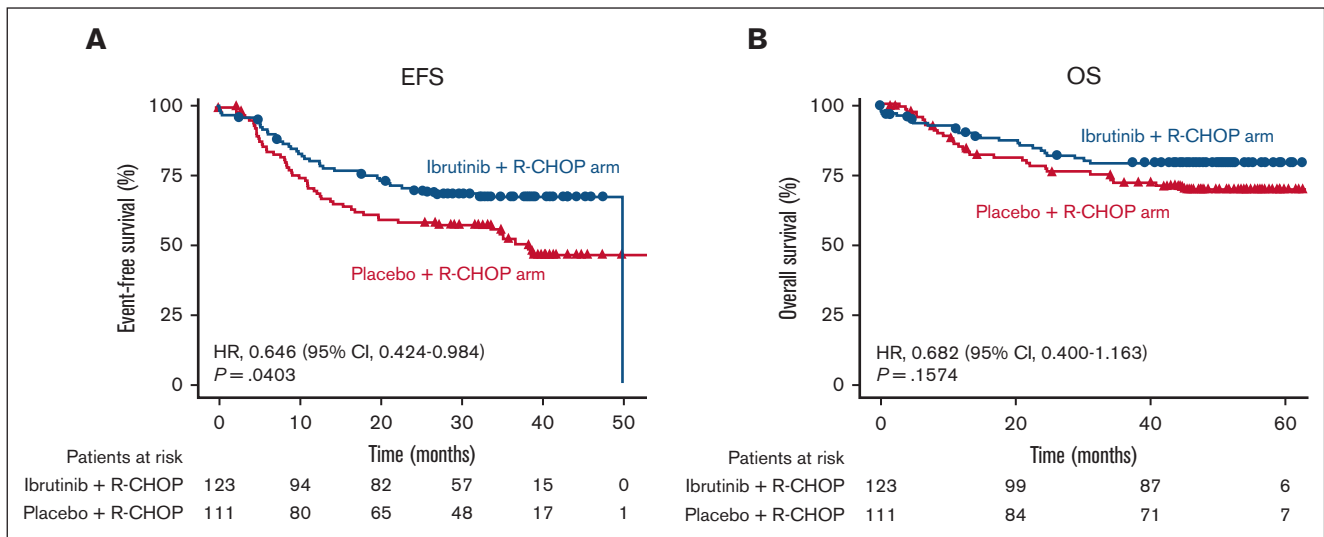
Figure 2. EFS and OS in patients with high *BCL2/MYC* coexpression by treatment arm. Overall EFS (A) and OS (B) in patients with high *BCL2/MYC* coexpression by RNA-seq in the placebo plus R-CHOP and ibrutinib plus R-CHOP arms.

When considering all patients with high *BCL2/MYC* coexpression, the EFS was superior in patients who received ibrutinib plus R-CHOP (n = 123) than in placebo plus R-CHOP (n = 111) (HR, 0.646; 95% CI, 0.424-0.984; *P* = .0403). A trend toward better OS was observed, but the difference was not significant (HR, 0.682; 95% CI, 0.400-1.163; *P* = .1574; Figure 2A-B).

The primary analysis of the PHOENIX study had shown that ibrutinib plus R-CHOP had improved EFS and OS over placebo plus R-CHOP (EFS: HR, 0.579; 95% CI, 0.380-0.881; *P* = .0099; OS: HR, 0.330; 95% CI, 0.162-0.673; *P* = .0013) in patients <60 years but not in patients ≥60 years, likely because of increased treatment toxicity in older patients.[28]

In this analysis, when the outcomes of patients with high *BCL2/MYC* expression were compared by age, both EFS (HR, 0.381; 95% CI, 0.193-0.752; *P* = .0039) and OS (HR, 0.234; 95% CI, 0.078-0.707; *P* = .0050) were improved in patients <60 years treated with ibrutinib plus R-CHOP (n = 47) rather than placebo plus R-CHOP (n = 50; Figure 3A-B). Interestingly, in patients ≥60 years with high *BCL2/MYC* coexpression (ibrutinib plus R-CHOP, n = 76; placebo plus R-CHOP, n = 61), there was no difference in EFS (HR, 0.924; 95% CI, 0.530-1.609; *P* = .7789) and OS (HR, 1.085; 95% CI, 0.562-2.095; *P* = .8068) between the treatment arms (Figure 3C-D). However, this result differed from that of the primary analysis, which reported a trend toward worse outcomes with ibrutinib plus R-CHOP than R-CHOP alone (EFS: HR, 1.228; 95% CI, 0.887-1.699; *P* = .2153; OS: HR, 1.440; 95% CI, 0.963-2.152; *P* = .0739) in patients ≥60 years.[28] This was possibly because of the increased benefit of ibrutinib addition to R-CHOP in the high *BCL2/MYC* expression group.

Single high *MYC* or *BCL2* expression and survival

There was no correlation with outcomes in patients with only a single high *MYC* or *BCL2* expression. Patients with high *MYC* but low *BCL2* expression and those with low *MYC* but high *BCL2* expression had similar EFS and OS in the ibrutinib plus R-CHOP (n = 73) and placebo plus R-CHOP (n = 76) arms (supplemental Figure 2A-D).

DLBCL subtypes and gene mutations associated with high *BCL2/MYC* coexpression by RNA-seq

We found that high *BCL2/MYC* coexpression was significantly associated with the ABC subtype of DLBCL (*P* < .0001), as well as the MCD genetic subtype (*P* = .0016; Table 2). The proportion of patients with DE lymphoma by RNA-seq was 35.7% in ABC vs 14.5% in GCB/unclassified DLBCL, and 44% in MCD vs 28.4% in non-MCD (supplemental Table 3). The A53 genetic subtype could not be inferred because aneuploidy assessment was not possible by methods used in this analysis. We also performed Fisher's exact test to determine which gene mutations were associated with the DE lymphoma status. Mutations in *MYD88*[L265P], *PIM1*, and *MYC* genes were significantly more common in DE lymphoma (all *P* < .05, odds ratio [OR] >1; Table 3) than in non-DE lymphoma. Mutations in other genes associated with ABC DLBCL, such as *CD79A*, *CD79B*, and *SYK*, were also associated with positive ORs, but the association with DE status was not significant. Mutations that were less common in DE lymphoma (OR <1) than in non-DE lymphoma included *BCL10*, *CYLD*, *NFKBIA*, *NFKB2*, *STAT3*, *PLCG2* (all *P* < .05), and *BTK* (*P* = .05) (Table 3).

Discussion

This analysis examined the clinical outcomes associated with high coexpression of *BCL2* and *MYC* using RNA-seq in the ibrutinib plus R-CHOP and placebo plus R-CHOP treatment arms from the phase 3 PHOENIX trial of non-GCB DLBCL. Our study effectively excluded patients with high *BCL2/MYC* expression owing to *BCL2* and *MYC* translocations (double hits), as these are infrequent and almost always occur in GCB DLBCL.[21,35] DE lymphoma is more common and is associated with the non-GCB subtype population in this trial.[20-22,36]

Several studies have reported high BCL2/MYC coexpression by IHC in 21% to 34% of DLBCL cases, with BCL2 and MYC high expression cutoffs of 50% to 70% and 40%, respectively.[20-22,36] In this analysis, we used median expression values for *BCL2* and *MYC* RNA expression, which corresponded to the BCL2 IHC
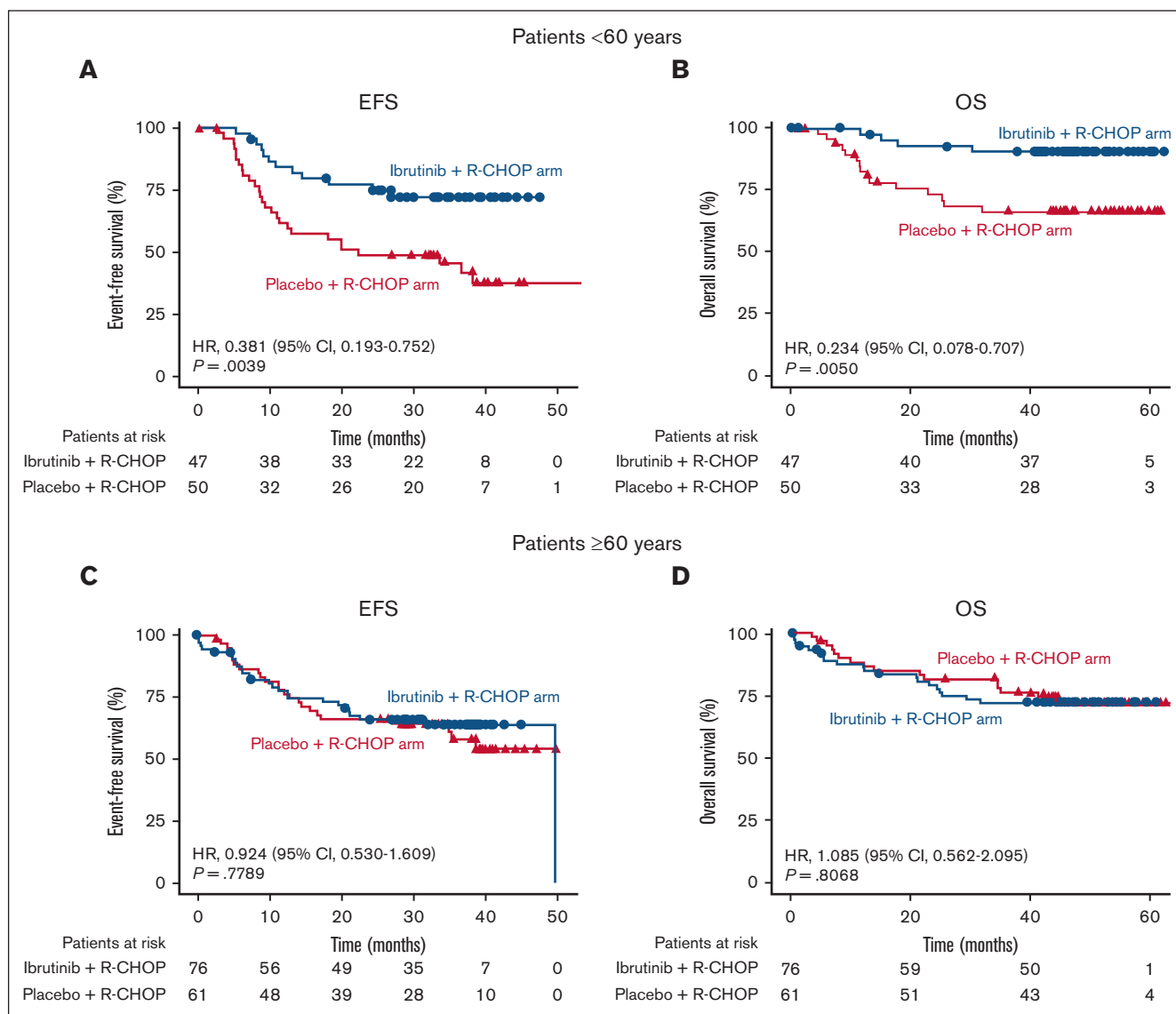
Figure 3. EFS and OS in patients with high *BCL2/MYC* coexpression by treatment arm and age. EFS and OS in patients with high *BCL2/MYC* coexpression by RNA-seq in the placebo plus R-CHOP and ibrutinib plus R-CHOP arms, aged <60 years (A,B) and ≥60 years (C,D).

cutoff of 50%. In the non-GCB population analyzed here, the frequency of high *BCL2/MYC* RNA coexpression among all patients tested was 30.5%, which is within the range reported for DE DLBCL measured by IHC in other studies.[20-22,36] Notably, a high correlation between BCL2 protein expression by IHC and *BCL2* RNA expression by RNA-seq was reported in experiments conducted using NHL cell lines,[37] as well as in tissue samples from patients with DLBCL of the ABC and GCB subtypes.[38,39]

Patients with DE DLBCL without translocations usually have DE DLBCL of ABC origin and have a poor prognosis with standard R-CHOP therapy.[3,22] Consistent with these reports, patients with high *BCL2/MYC* coexpression treated with R-CHOP in this study had worse EFS and OS than those without high *BCL2/MYC* coexpression (*P* = .0010 and *P* = .0055, respectively). However, when patients with high *BCL2/MYC* coexpression were treated

with ibrutinib plus R-CHOP, there was no difference in survival compared with patients without high *BCL2/MYC* coexpression, suggesting that the negative prognostic impact of high *BCL2/MYC* coexpression may have been overcome by ibrutinib.

When considering all patients with high *BCL2/MYC* coexpression, there was a significantly better EFS (and a trend toward improved OS) in patients who received ibrutinib plus R-CHOP than in those who received placebo plus R-CHOP. In contrast, there was no EFS or OS benefit of ibrutinib when considering the ITT population from the PHOENIX study.[28] Our results suggest that high *BCL2/MYC* coexpression may identify a subset of non-GCB DLBCL that particularly benefit from the addition of ibrutinib to R-CHOP. However, both the PHOENIX study and our subset analysis of patients with high *BCL2/MYC* coexpression showed that the benefit of ibrutinib in addition to R-CHOP was stronger in younger patients.

Table 2. A pairwise analysis of the association between DE lymphoma status and DLBCL subtypes*†‡

DE vs ABC calls (RNA-seq vs HTG EdgeSeq), n = 705				
Subtypes, n (%)	**DE, n = 217**	**OR (95% CI)**	**P value**	**Non-DE, n = 488**
ABC	193 (88.9)	3.26 (2.02-5.45)	<.0001	347 (71.1)
GCB/unclassified	24 (11.1)	-	-	141 (28.9)
DE vs MCD/N1/BN2 calls (RNA-seq vs LymphGen), n = 765				
Subtypes, n (%)	**DE, n = 234**	**OR (95% CI)**	**P value**	**Non-DE, n = 531**
MCD	48 (20.5)	0.50 (0.33-0.78)	.0016	61 (11.5)
Non-MCD	186 (79.5)	-	-	470 (88.5)
N1	11 (4.7)	0.67 (0.29-1.61)	.3034	17 (3.2)
Non-N1	223 (95.3)	-	-	514 (96.8)
BN2	10 (4.3)	1.63 (0.77-3.75)	.2472	36 (6.8)
Non-BN2	224 (95.7)	-	-	495 (93.2)
ABC vs MCD/N1/BN2 calls (HTG EdgeSeq vs LymphGen), n = 709				
Subtypes, n (%)	**ABC, n = 543**	**OR (95% CI)**	**P value**	**GCB/unclassified, n = 166**
MCD	101 (18.6)	0.08 (0.02-0.25)	<.0001	3 (1.8)
Non-MCD	442 (81.4)	-	-	163 (98.2)
N1	22 (4.1)	0.59 (0.14-1.76)	.4785	4 (2.4)
Non-N1	521 (95.9)	-	-	162 (97.6)
BN2	34 (6.3)	1.17 (0.54-2.38)	.7186	12 (7.2)
Non-BN2	509 (93.7)	-	-	154 (92.8)

A53, aneuploid with *TP53* inactivation.

*Any deviations from the total number of patients in the ITT population (n = 838), the RNA-seq–surveyed subset (766 patients with DE/non-DE calls), the HTG EdgeSeq-surveyed subset (747 patients with ABC/GCB/Unclassified calls), or the subset with LymphGen calls (773 patients with MCD/N1/BN2/other calls), result from a lack of complete overlap between patients surveyed with the different assays.

†A53 subtype could not be inferred as assessing aneuploidy was not allowed by methods used in the analysis.

‡*P* < .01 for the following pairwise Fisher's exact tests: DE vs MCD, DE vs ABC, and MCD vs ABC. All other Fisher's exact results had *P* > .20.

In this analysis, the proportion of patients with high *BCL2/MYC* coexpression by RNA-seq was similar between younger (<60 years, 30.6%) and older (≥60 years, 30.5%) patients. This result differs from the previously reported data showing a higher frequency of BCL2/MYC protein coexpression in older patients (68 years).[20]

Although DE lymphomas are not a distinct clinicopathological entity in the revised World Health Organization classification,[2] patients with DLBCL featuring high MYC/BCL2 coexpression by IHC have been reported to be more likely to have advanced disease stage, high Ki67 proliferative index scores, poor ECOG PS scores, multiple extranodal disease sites, and high-risk R-IPI scores.[22,40,41] In this analysis, we did not observe any major differences in the clinicopathological characteristics of patients with vs without high *BCL2/MYC* coexpression by RNA-seq, except for a slightly higher proportion of patients in the high *BCL2/MYC* group having elevated lactate dehydrogenase levels. This result may be a reflection of the non-GCB enriched population in this study, with more ABC subtypes than those included in previous studies (71% in this analysis vs 38%-48% in previous studies[22,40]).

The current analysis was based on the hypothesis that *BCL2/MYC* DE lymphomas would have, on average, greater NF-κB signaling activity, and that patients with disease driven primarily by NF-κB signaling would be more likely to respond to ibrutinib. In the traditional gene expression-based ABC/GCB subtyping scheme, the ABC subtype is generally driven more by NF-κB signaling.[9,42] The same can be said for the MCD subtype in a newer genetic classification system,[11] and presumably the very similar C5 cluster in an alternative genetic classification,[14] enriched for *MYD88^L265P* and *CD79B* mutations. Moreover, gene mutations identified in this analysis as frequently associated with DE DLBCL (*MYD88^L265P*, *PIM1, CD79B)* also correspond to mutations described for the MYD88 cluster in yet another 5-molecular subtype DLBCL classification based on DNA sequencing and Bernoulli mixture-model clustering.[15]

Recent evidence suggests that patients with DLBCL of the MCD genetic subtype benefit from the addition of ibrutinib to R-CHOP.[12] One perspective of the results reported here is that the *BCL2/MYC* DE population may be enriched for ABC and/or MCD subtypes of DLBCL. Supporting this statement, we found evidence of an association between high *BCL2/MYC* coexpression by RNA-seq and the ABC subtype (*P* < .0001) or MCD genetic subtype (*P* = .0016). We also identified gene mutations that were more frequently found in patients with high *BCL2/MYC* coexpression, such as *MYD88^L265P*, *PIM1, MYC* (*P* < .05 for all) and *CD79A, CD79B,* and *SYK* (not significant), several of which have recently been reported to be associated with the MCD genetic subtype.[11]

The main limitation of this analysis is that because of the insufficient amount of tissue for analysis, we used an RNA-seq method rather than IHC to measure the expression of *BCL2/MYC*, although IHC was performed on available patient samples to partially validate the RNA-seq data. Although this method has not yet been fully

Table 3. Gene mutations more frequent (OR >1) or less frequent (OR <1) in patients with DE lymphoma vs non-DE lymphoma*

Gene	DE, n		Non-DE, n		P value	OR	95% CI
	WT	Mut	WT	Mut			
MYD88[L265P]	176	46	428	51	.0006	2.1907	1.38-3.47
CD79A	212	10	468	11	.1505	2.0047	0.75-5.29
MYC	198	24	450	29	.0316	1.8791	1.02-3.44
ERBB4	213	9	468	11	.2238	1.7960	0.65-4.85
SYK	208	14	461	18	.1718	1.7224	0.78-3.74
PIM1	152	70	378	101	.0034	1.7222	1.18-2.50
MET	203	19	453	26	.1357	1.6295	0.83-3.14
TRAF3	212	10	465	14	.2745	1.5657	0.61-3.86
TRAF4	211	11	463	16	.2987	1.5077	0.62-3.53
WHSC1	198	24	442	37	.1953	1.4472	0.81-2.56
CD79B	175	47	395	84	.2536	1.2625	0.83-1.91
KMT2D	142	80	330	149	.1957	1.2473	0.88-1.77
TNFAIP3	193	29	396	83	.1834	0.7172	0.44-1.15
EP300	192	30	388	91	.0855	0.6666	0.41-1.06
NOTCH1_N	202	20	416	63	.1317	0.6542	0.36-1.13
MYD88_nonL265P	208	14	432	47	.1498	0.6190	0.31-1.18
STAT3	210	12	430	49	.0429	0.5019	0.24-0.98
CARD11_nonCC	217	5	457	22	.2039	0.4791	0.14-1.32
PLCG2	212	10	436	43	.0450	0.4787	0.21-0.99
TRAF2	218	4	461	18	.2435	0.4704	0.11-1.45
TXK	216	6	452	27	.1235	0.4655	0.15-1.17
ITK	219	3	463	16	.2091	0.3968	0.07-1.41
TRAF5	219	3	463	16	.2091	0.3968	0.07-1.41
BTK	217	5	452	27	.0515	0.3862	0.11-1.04
VRK2	218	4	457	22	.0851	0.3816	0.09-1.14
CSK	218	4	456	23	.0589	0.3642	0.09-1.08
NFKB2	217	5	450	29	.0360	0.3580	0.11-0.95
CYLD	217	5	447	32	.0170	0.3223	0.10-0.85
MDM2	219	3	459	20	.0662	0.3148	0.06-1.08
NFKBIA	219	3	457	22	.0297	0.2849	0.05-0.96
BCL10	218	4	448	31	.0081	0.2655	0.07-0.76

mut, mutated; WT, wild-type.
*List of genes for which P < .3.

validated for the characterization of DE lymphoma, it has been used for biomarker identification in relapsed or refractory non-GCB DLBCL treated with zanubrutinib[43] and may prove valuable in cases where insufficient biopsy tissue precludes protein expression analysis. Another limitation was that this analysis did not evaluate BCL2/MYC rearrangements or other gene aberrations that may be associated with high BCL2/MYC RNA coexpression.

Our data suggested that high BCL2/MYC RNA coexpression may identify a subset of non-GCB DLBCL that is highly responsive to ibrutinib treatment. Ibrutinib plus R-CHOP improved the EFS in patients with BCL2/MYC high-expressing non-GCB DLBCL, as well as the EFS and OS in the subset aged <60 years—a finding that warrants further investigation.

Acknowledgments

Authorship

Contribution: P.W.M.J., S.B., B.H., L.H.S., and W.H.W. conceptualized the study; P.W.M.J., S.B., B.H., S.M.S., S. Sun, J.V., L.H.S., and W.H.W. were involved in the investigation; and all authors analyzed the data, contributed to the writing of the manuscript, and approved the final draft for submission.

Conflict-of-interest disclosure: P.W.M.J. served as a consultant or in an advisory role for Janssen, received research funding from Epizyme and Janssen, and honoraria from Bristol-Myers Squibb, Takeda, Novartis, Celgene, Janssen, Epizyme, Kite, Genmab, Kymera, Oncimmune, Immunocore, and Incyte. S.B. owns stock in Johnson & Johnson, Gilead Sciences, Celgene, Vertex, and AbbVie and is a former employee of the Janssen Pharmaceutical Companies of Johnson & Johnson, currently retired. B.H., S.M.S., S. Sun, A.J.S., S. Srinivasan, and J.V. are employees of the Janssen Pharmaceutical Companies of Johnson & Johnson. S.M.S., S. Sun, A.J.S., and J.V. own stock in Johnson & Johnson. L.H.S. served as a consultant or in an advisory role for Celgene, AbbVie, Seattle Genetics, TG Therapeutics, Janssen, Amgen, Roche/Genentech, Gilead Sciences, Lundbeck, Apobiologix, Karyopharm Therapeutics, Kite Pharma, Merck, Takeda Pharmaceuticals, TEVA Pharmaceuticals Industries, and TG Therapeutics, received research fundings from Roche/Genentech and honoraria from Amgen, Apobiologix, AbbVie, Celgene, Gilead Sciences, Incyte Janssen-Ortho, Karyopharm Therapeutics, Kite Pharma, Lundbeck, Merck, Roche/Genentech, Sandoz, Seattle Genetics, Takeda Pharmaceuticals, TEVA Pharmaceuticals Industries, and TG Therapeutics. W.H.W. has no competing financial interest.

ORCID profile: P.W.M.J., 0000-0003-2306-4974.

Correspondence: Peter W. M. Johnson, Centre for Cancer Immunology, University of Southampton, Southampton General Hospital, Southampton SO16 6YD, United Kingdom; email: johnsonp@soton.ac.uk.

References

1. Padala SA, Kallam A. Diffuse large B cell lymphoma. Treasure Island, FL: StatPearls; 2021.

2. Swerdlow SH, Campo E, Pileri SA, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood. 2016; 127(20):2375-2390.

3. Scott DW, Mottok A, Ennishi D, et al. Prognostic significance of diffuse large B-cell lymphoma cell of origin determined by digital gene expression in formalin-fixed paraffin-embedded tissue biopsies. *J Clin Oncol*. 2015;33(26):2848-2856.

4. National Comprehensive Cancer Network (NCCN®). Clinical practice guidelines in oncology: B-cell lymphomas version 5.2022. Accessed 27 July 2022. https://www.nccn.org/guidelines/guidelines-detail?category=1&id=1480

5. Vaidya R, Witzig TE. Prognostic factors for diffuse large B-cell lymphoma in the R(X)CHOP era. *Ann Oncol*. 2014;25(11):2124-2133.

6. Alizadeh AA, Eisen MB, Davis RE, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature*. 2000;403(6769): 503-511.

7. Rosenwald A, Wright G, Chan WC, et al. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. *N Engl J Med*. 2002;346(25):1937-1947.

8. Hans CP, Weisenburger DD, Greiner TC, et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. *Blood*. 2004;103(1):275-282.

9. Davis RE, Brown KD, Siebenlist U, Staudt LM. Constitutive nuclear factor kappaB activity is required for survival of activated B cell-like diffuse large B cell lymphoma cells. *J Exp Med*. 2001;194(12):1861-1874.

10. Lenz G, Wright G, Dave SS, et al. Stromal gene signatures in large-B-cell lymphomas. *N Engl J Med*. 2008;359(22):2313-2323.

11. Schmitz R, Wright GW, Huang DW, et al. Genetics and pathogenesis of diffuse large B-cell lymphoma. *N Engl J Med*. 2018;378(15):1396-1407.

12. Wilson WH, Wright GW, Huang DW, et al. Effect of ibrutinib with R-CHOP chemotherapy in genetic subtypes of DLBCL. *Cancer Cell*. 2021;39(12): 1643-1653.e3.

13. Wright GW, Huang DW, Phelan JD, et al. A probabilistic classification tool for genetic subtypes of diffuse large B cell lymphoma with therapeutic implications. *Cancer Cell*. 2020;37(4):551-568.e14.

14. Chapuy B, Stewart C, Dunford AJ, et al. Molecular subtypes of diffuse large B cell lymphoma are associated with distinct pathogenic mechanisms and outcomes. *Nat Med*. 2018;24(5):679-690.

15. Lacy SE, Barrans SL, Beer PA, et al. Targeted sequencing in DLBCL, molecular subtypes, and outcomes: a Haematological Malignancy Research Network report. *Blood*. 2020;135(20):1759-1771.

16. Vaux DL, Cory S, Adams JM. Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. *Nature*. 1988; 335(6189):440-442.

17. Adams JM, Harris AW, Pinkert CA, et al. The c-myc oncogene driven by immunoglobulin enhancers induces lymphoid malignancy in transgenic mice. *Nature*. 1985;318(6046):533-538.

18. Nguyen L, Papenhausen P, Shao H. The role of c-MYC in B-cell lymphomas: diagnostic and molecular aspects. *Genes (Basel)*. 2017;8(4):116.

19. Lu TX, Fan L, Wang L, et al. MYC or BCL2 copy number aberration is a strong predictor of outcome in patients with diffuse large B-cell lymphoma. *Oncotarget*. 2015;6(21):18374-18388.

20. 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(28):3460-3467.

21. 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(28):3452-3459.

22. 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(20):4021-4031;quiz 4250.

23. *IMBRUVICA® (ibrutinib) [prescribing information]. Pharmacyclics LLC and Janssen Biotech, Inc.* 2019.

24. Wilson WH, Young RM, Schmitz R, et al. Targeting B cell receptor signaling with ibrutinib in diffuse large B cell lymphoma. *Nat Med*. 2015;21(8):922-926.

25. Davis RE, Ngo VN, Lenz G, et al. Chronic active B-cell-receptor signalling in diffuse large B-cell lymphoma. *Nature*. 2010;463(7277):88-92.

26. Younes A, Thieblemont C, Morschhauser F, et al. Combination of ibrutinib with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) for treatment-naive patients with CD20-positive B-cell non-Hodgkin lymphoma: a non-randomised, phase 1b study. *Lancet Oncol*. 2014; 15(9):1019-1026.

27. Schaffer M, Chaturvedi S, Davis C, et al. Activity of ibrutinib plus R-CHOP in diffuse large B-cell lymphoma: response, pharmacodynamic, and biomarker analyses of a phase Ib study. *Cancer Treat Res Commun*. 2020;25:100235.

28. Younes A, Sehn LH, Johnson P, et al. Randomized phase III trial of ibrutinib and rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone in non-germinal center B-cell diffuse large B-cell lymphoma. *J Clin Oncol*. 2019;37(15):1285-1295.

29. Wang WG, Liu ZB, Jiang XN, Lee J, Zhou XY, Li XQ. MYC protein dysregulation is driven by BCR-PI3K signalling in diffuse large B-cell lymphoma. *Histopathology*. 2017;71(5):778-785.

30. Xia Y, Zhang X. The spectrum of MYC alterations in diffuse large B-cell lymphoma. *Acta Haematol*. 2020;143(6):520-528.

31. Bogusz AM, Kovach AE, Le LP, Feng D, Baxter RHG, Sohani AR. Diffuse large B-cell lymphoma with concurrent high MYC and BCL2 expression shows evidence of active B-cell receptor signaling by quantitative immunofluorescence. *PLoS One*. 2017;12(2):e0172364.

32. Cheson BD, Pfistner B, Juweid ME, et al. Revised response criteria for malignant lymphoma. *J Clin Oncol*. 2007;25(5):579-586.

33. Balasubramanian S, Wang S, Major C, et al. Comparison of immunohistochemistry and gene expression profiling subtyping for diffuse large B-cell lymphoma in the phase III clinical trial of R-CHOP +/- ibrutinib. *Br J Haematol*. 2021;194(1):83-91.

34. Balasubramanian S, Hodkinson B, Schuster SJ, et al. Identification of a genetic signature enriching for response to ibrutinib in relapsed/refractory follicular lymphoma in the DAWN phase 2 trial. *Cancer Med*. 2022;11(1):61-73.

35. Le Gouill S, Talmant P, Touzeau C, et al. The clinical presentation and prognosis of diffuse large B-cell lymphoma with t(14;18) and 8q24/c-MYC rearrangement. *Haematologica*. 2007;92(10):1335-1342.

36. Batlle-Lopez A, Gonzalez de Villambrosia S, Francisco M, et al. Stratifying diffuse large B-cell lymphoma patients treated with chemoimmunotherapy: GCB/non-GCB by immunohistochemistry is still a robust and feasible marker. *Oncotarget*. 2016;7(14):18036-18049.

37. Punnoose E, Peale FV, Szafer-Glusman E, et al. BCL2 expression in first-line diffuse large B-cell lymphoma identifies a patient population with poor prognosis. *Clin Lymphoma Myeloma Leuk*. 2021;21(4):267-278.e10.

38. Iqbal J, Neppalli VT, Wright G, et al. BCL2 expression is a prognostic marker for the activated B-cell-like type of diffuse large B-cell lymphoma. *J Clin Oncol*. 2006;24(6):961-968.

39. Shen Y, Iqbal J, Huang JZ, Zhou G, Chan WC. BCL2 protein expression parallels its mRNA level in normal and malignant B cells. *Blood*. 2004;104(9):2936-2939.

40. Pena C, Villegas P, Cabrera ME. Double or triple-expressor lymphomas: prognostic impact of immunohistochemistry in patients with diffuse large B-cell lymphoma. *Hematol Transfus Cell Ther*. 2020;42(2):192-193.

41. Riedell PA, Smith SM. Double hit and double expressors in lymphoma: definition and treatment. *Cancer*. 2018;124(24):4622-4632.

42. Staudt LM. Oncogenic activation of NF-kappaB. *Cold Spring Harb Perspect Biol*. 2010;2(6):a000109.

43. Yang H, Li Y, Oh SY, et al. Biomarker identification in relapsed/refractory non-germinal center B-cell–like diffuse large B-cell lymphoma treated with zanubrutinib. *HemaSphere*. 2020;4(S1):584.