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ORIGINAL ARTICLE

# Zanubrutinib plus R-CHOP for the treatment of newly diagnosed double-expressor lymphoma: A phase 2 clinical study

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## Abstract

**Background:** Double-expressor lymphoma (DEL) has a poorer prognosis than other subtypes of diffuse large B-cell lymphoma (DLBCL). This study is a multicenter, prospective, single-arm, phase 2 clinical study initiated by investigators to evaluate the efficacy and safety of combined zanubrutinib with R-CHOP, which includes rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone for patients with DEL (stage II or more), as well as to explore factors related to efficacy preliminarily.

**Methods:** From November 2020 to July 2022, 48 newly diagnosed patients were enrolled. All patients received twice-daily oral zanubrutinib (160 mg) for 6 months and standardized R-CHOP regimen for six to eight cycles.

**Results:** The objective response rate (ORR) was 89.6%, with a complete response rate (CRR) of 83.3%. The median follow-up was 29.3 months. The median progression-free survival (PFS) and overall survival (OS) were not reached. The PFS and OS were 81.25% and 93.75% at 2 years, respectively. Grade ≥3 adverse events (AEs) were reported in 23 of 48 (47.9%) patients. Next-generation sequencing (NGS) results of 33 patients showed that *TP53*, *MYD88*, and *PIM1* were the most common mutated gene. Multivariate analysis revealed that *BCL-6* gene rearrangement was an adverse prognostic factor for both PFS (hazard ratio [HR], 0.247; 95% confidence article [CI], 0.068–0.9; $p = .034$) and OS (HR, 0.057; 95% CI, 0.006–0.591; $p = .016$), whereas the number of extranodal involvements also significantly influenced OS (HR, 15.12; 95% CI, 1.07–213.65; $p = .044$).

**Conclusions:** Zanubrutinib in combination with R-CHOP is an effective option for DEL patients, and the toxicity of zanubrutinib is entirely acceptable for patients.

Xia Yin and Qiang He contributed equally to this article.

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KEYWORDS
BCL2, BTKi, double-expressor lymphoma, MYC, zanubrutinib

## INTRODUCTION

The double-expressor lymphoma (DEL) refers to the simultaneous expression of MYC and BCL2 protein in lymphoma tissue through immunohistochemical (IHC) staining, without the presence of *MYC* and *BCL2* gene rearrangements or translocations. In 2016, the World Health Organization established cutoff values of positivity ≥40% for MYC and ≥50% for BCL2 by IHC for DEL.[1] DEL accounts for approximately 20%–30% of newly diagnosed diffuse large B-cell lymphoma (DLBCL).[2] The standard frontline treatments for DLBCL are rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) protocols. Unfortunately, this protocol is associated with a poor prognosis in patients with DEL and the reported 5-year overall survival (OS) was 39%.[3]

For the above reasons, many attempts have also been made, such as adjusted chemotherapy intensity, first-line autologous Hematopoietic Stem Cell Transplantation (auto-HSCT), and combining with new targeted agents (R-CHOP plus X).[4–6] Nevertheless, less efficacy and more toxic effects were found. For example, in the Phoenix study, an analysis of patients with high BCL2/MYC expression indicated that, compared to placebo plus R-CHOP treatment, the combination of ibrutinib and R-CHOP (IR-CHOP) tended to improve progression-free survival (PFS) in patients under 60 years old ($p = .0381$).[7] However, in patients 60 years and older, the IR-CHOP was associated with increased toxicity, leading to a higher discontinuation rate and reduced efficacy.

As drug research advances, the second-generation Bruton tyrosine kinase inhibitor (BTKi) acalabrutinib has demonstrated outstanding efficacy. In the ACCEPT trial, acalabrutinib combined with R-CHOP (AR-CHOP) was studied as a frontline treatment for DLBCL, achieving an objective response rate (ORR) of 95% and a metabolic complete response (mCR) of 82%.[8] The ongoing ESCALADE phase 3 trial is comparing the efficacy of AR-CHOP versus R-CHOP alone in untreated non-GCB DLBCL patients.[9]

Zanubrutinib is a second-generation BTKi with higher target occupancy than ibrutinib and fewer off-target effects.[10] Moreover, zanubrutinib may have better clinical efficacy, thereby increasing patient benefit.

The optimal treatment regimen for DEL patients has not yet been determined. In our phase 2 study, we aimed to evaluate the efficacy and safety of zanubrutinib combined with R-CHOP (ZR-CHOP) in patients with DEL (especially in elderly patients).

## MATERIALS AND METHODS

### Study design

This open-label, multicenter, single-arm, prospective phase 2 study aims to evaluate the efficacy and safety of ZR-CHOP in newly diagnosed DEL patients. It was conducted at five hospitals (Shandong Cancer Hospital and Institute, Shandong First Medical University and Shandong Academy of Medical Sciences, Qilu Hospital of Shandong University, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Shengli Oilfield Central Hospital, and Jining First People's Hospital). All patients signed and dated a written informed consent form at the first screening visit. This study was approved by an ethics committee and adhered to the principles of the Helsinki Declaration for Good Clinical Practice and its subsequent amendments (ethical approval: SDZLEC2020-129-02). The institutional review boards and/or independent ethics committees reviewed and approved the protocol and informed consent forms.

### Patients and eligibility

The study included patients diagnosed with de novo DEL from November 2020 to July 2022. The diagnosis was confirmed by IHC, which identified DEL: the possibility of MYC ≥40% and BCL-2 >50%. Fluorescence in situ hybridization (FISH) testing was also conducted to exclude rearrangements and translocations of the two genes. Thirty-three patients were tested with the same next-generation sequencing (NGS), which was performed on genomic DNA isolated from formalin-fixed paraffin-embedded (FFPE) tumor samples using the NextSeq platform (Illumina, San Diego, California). Hybrid capture was employed to analyze the whole exonic sequences of 43 genes associated with DLBCL (*ITPKB*, *JAK2*, *KMT2C*, *KMT2D*, *MEF2B*, *MFHAS1*, *MYC*, *MYD88*, *NOTCH1*, *NOTCH2*, *PAX5*, *PIM1*, *SGK1*, *SOCS1*, *STAT3*, *STAT6*, *TET2*, *TNFAIP3*, *TNFRSF14*, *TP53*, *XPO1*, *ARID1B*, *ATM*, *B2M*, *BCL10*, *BCL2*, *BCL6*, *BTG1*, *CARD11*, *CCND3*, *CD58*, *CD79A*, *CD79B*, *CDKN2A*, *PRDM1*, *CIITA*, *CREBBP*, *EP300*, *EPHA7*, *EZH2*, *FAS*, *GNA13*, and *IRF8*). All variants were detected with >99% confidence based on allele frequency and amplicon coverage, with an average sequencing depth of coverage of >500 and an analytic sensitivity of 5%. A minimum variant allele frequency (VAF) of 5% was used to call single nucleotide variants.

### Treatment

The patients received zanubrutinib (160 mg by mouth, twice daily) for 6 months and rituximab (375 mg/m$^2$, day 0), cyclophosphamide (750 mg/m$^2$, day 1), doxorubicin (50 mg/m$^2$, day 1), vincristine (1.4 mg/m$^2$, day 1), and prednisone (100 mg, days 1–5) in repeated 21-day cycles for six to eight cycles.[11–13]

### Efficacy and safety assessment

The study's primary end points are PFS and OS. Secondary end points include complete response rate (CRR), ORR (complete response [CR] plus partial response [PR]), and adverse events (AEs).

Exploratory end points investigate the impact of clinical characteristics, biological markers, genetic abnormalities, and other prognostic factors on treatment efficacy and survival. Patients will undergo positron emission tomography and contrast computed tomography (PET-CT) evaluation before treatment, after four and eight cycles of treatment, and will have efficacy assessment with enhanced CT scans of the neck, chest, abdomen, and pelvis at two and six cycles as a substitute for PET-CT. Treatment responses were evaluated according to Lugano criteria.[14] The investigator assessed the severity of AEs reported during the study. When applicable, AEs were assessed and graded based on the National Cancer Institute Common Terminology Criteria for Adverse Events v5.0 guidelines.

## Statistical analysis

PFS was defined as the time from the date of diagnosis to the date of disease progression/relapse, death, or last follow-up. OS was calculated as the time from diagnosis to the day of death due to any cause or last follow-up. Survival was calculated with the Kaplan–Meier method, and the significance between subgroups was calculated using the log-rank test. In univariate and multivariate analysis, the Cox proportional hazards model was used to identify the factors that were significantly associated with PFS or OS. Variables with statistical significance ($p < .05$) were included in the multivariate analysis. Descriptive statistics for continuous data included mean, median, maximum, and minimum values. Frequency counts and percentages were used for categorical data. This study used SPSS 26 statistical analysis software for statistical calculations. Survival curves and heat maps were generated using R software. $p < 0.05$ indicates the statistical significance of the differences. The sample size was estimated that 2-year PFS rate for the treatment in this study will be 70%, compared to the 50% in the historical control. If 50 subjects (15 events) are enrolled within 12 months, and the analysis of 2-year PFS rate is conducted 12 months after the last patient is enrolled, with an expected dropout rate of 20%, it will have 85% power that the lower limit of the 90% confidence interval for 2-year PFS rate will be higher than the historical control of 50%.

# RESULTS

## Patients and baseline characteristics

From November 2020 to July 2022, 48 patients were enrolled in the study (38, six, two, one, and one patient from Shandong Cancer Hospital and Institute, Shandong First Medical University and Shandong Academy of Medical Sciences, Qilu Hospital of Shandong University, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Shengli Oilfield Central Hospital, and Jining First People's Hospital, respectively) and detailed in Figure 1. The demographic and baseline characteristics of these patients are shown in Table 1. Of the patients enrolled, 29 were male and 19 were female. The median age was 59 years (range, 17–76). We recruited only patients with stages II, III, and IV. The majority of them had predominantly non-GCB typing (89.6%). Additionally, 33 patients underwent NGS testing for *TP53*, with 12 patients showing *TP53* mutation. Forty-four patients tested for *BCL-6* FISH assay, and eight (18.2%) patients had *BCL-6* rearrangement.

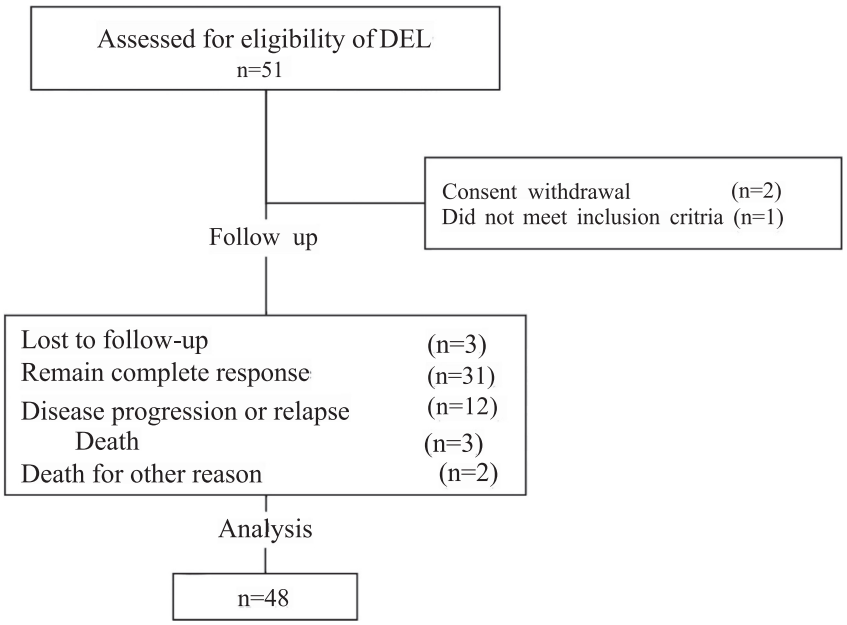


**FIGURE 1** The CONSORT diagram "Death" in the diagram means patients died for disease progression or relapse. CONSORT indicates Consolidated Standards of Reporting Trials.

**TABLE 1** Baseline characteristics of total patients.

| | No. | % |
|---|---|---|
| Gender | 48 | |
| Male | 29 | 60.4 |
| Female | 19 | 39.6 |
| Age (years) | 48 | |
| Median (range) | 59 (17–76) | |
| <60 | 26 | 54.2 |
| ≥60 | 22 | 45.8 |
| No. of extranodal involvement | 48 | |
| 0-1 | 30 | 62.5 |
| ≥2 | 18 | 37.5 |
| LDH | 41 | |
| Normal | 20 | 48.8 |
| Rise | 21 | 51.2 |
| Ann Arbor stage | 48 | |
| II | 22 | 45.8 |
| III–IV | 26 | 54.2 |
| IPI risk group | 48 | |
| 0–2 | 28 | 58.3 |
| 3–5 | 20 | 41.7 |
| Cell of origin (Hans) | 48 | |
| Non-GCB | 43 | 89.6 |
| GCB | 5 | 10.4 |
| *BCL6* rearrangement | 44 | |
| *BCL-6*– | 36 | 81.8 |
| *BCL-6*+ | 8 | 18.2 |
| *BCL-2* rearrangement | 44 | |
| *BCL-2*– | 41 | 93.2 |
| *BCL-2*+ | 3 | 6.8 |
| *TP53* | 33 | |
| Wild | 21 | 63.6 |
| Mutation | 12 | 36.4 |

Abbreviations: *BCL-6*+, *BCL-6* rearrangement positive; *BCL-2*+, *BCL-2* rearrangement positive; GCB, germinal center B-cell type; IPI, International Prognostic Index; LDH, lactate dehydrogenase; non-GCB, nongerminal center B-cell type.

## Survival/efficacy

At the end of four treatment cycles, PET-CT examination for efficacy evaluation revealed that 37 (77.1%) patients achieved CR, and 10 (20.8%) patients achieved PR. ORR reached 97.9% and CRR reached 77.1%. After completing eight cycles of treatment, our efficacy evaluation indicated that 40 patients (83.3%) achieved CR, three patients (6.3%) achieved PR, and three patients (6.3%) had progressive disease (PD). The ORR and CRR were 89.6% and 83.3%, respectively.

As of the follow-up conducted until March 29, 2024, the median follow-up was 29.3 (2.7–43.3) months. Three patients were lost to follow-up. Thirty-one patients (64.6%) remain in CR, with a total of 12 patients (25.0%) experiencing PD, of which three patients died due to PD after six cycles of treatment. One patient died of cardiovascular disease at the end of the second cycle of treatment at a nonresearch clinical center, and an autopsy was not performed, so it cannot be ruled out whether the death was caused by sudden death due to AEs. Additionally, one patient died because of the COVID-19 infection after eight cycles of treatment and his efficacy evaluation is CR. The median PFS and OS were not reached. The PFS and OS were 81.25% and 93.75% at 2 years, respectively. The PFS and OS curves are shown in Figure 2A,B. All exhibited PR or CR at the first assessment Figure 3.

## Univariate and multivariate analysis of survival

Cox univariate and multivariate models concerning the PFS and OS of the patients were performed as summarized in Tables 2 and 3. We performed a univariate analysis on the impacts of age, gender, number of extranodal involvement, lactate dehydrogenase (LDH), stage, International Prognostic Index (IPI), cell of origin, *BCL-6*, *TP53*, and Eastern Cooperative Oncology Group (ECOG) for PFS and OS. Multivariate analysis revealed that *BCL-6* gene rearrangement was an adverse prognostic factor for both PFS (HR, 0.247; 95% CI, 0.068–0.9; $p = .034$) and OS (HR, 0.057; 95% CI, 0.006–0.591; $p = .016$), whereas the number of extranodal involvements also significantly influenced OS (HR, 15.12; 95% CI,1.07–213.65; $p = .044$). Factors associated with PFS and OS are shown in Figures 4A–F.

## Biomarker substudy

We created a heatmap for 33 patients who were tested for the same genes. The results showed that the genes with the highest mutation rates were *TP53* (36%), *MYD88* (33%), and *PIM1* (33%). Among the patients with *TP53* mutations, four had *TP53* oncogenic gene mutations, with only one patient showing PD at the sixth cycle (mutation site: NM_000546.844C>T [p.R282W] exon 8, mutation frequency: 45.50%). The remaining three patients maintained CR at the end of the last follow-up. All seven patients with possible oncogenic mutations maintained CR at the end of follow-up. Of the nine patients with *MYD88* oncogenic gene mutations, one experienced PD (mutation site: NM_002468.4.794T>C [p.L265P] exon 5). Seven patients maintained CR at the end of follow-up. One patient died of COVID-19 (mutation frequency of 14.69%). The heatmap is shown in Figure 5.

*STAT3* (HR, 31.49; 95% CI, 1.97–503.58, $p = 0.015$) and *PAX5* (HR, 15.49; 95% CI, 1.40–170.89; $p = .025$) mutation associated with

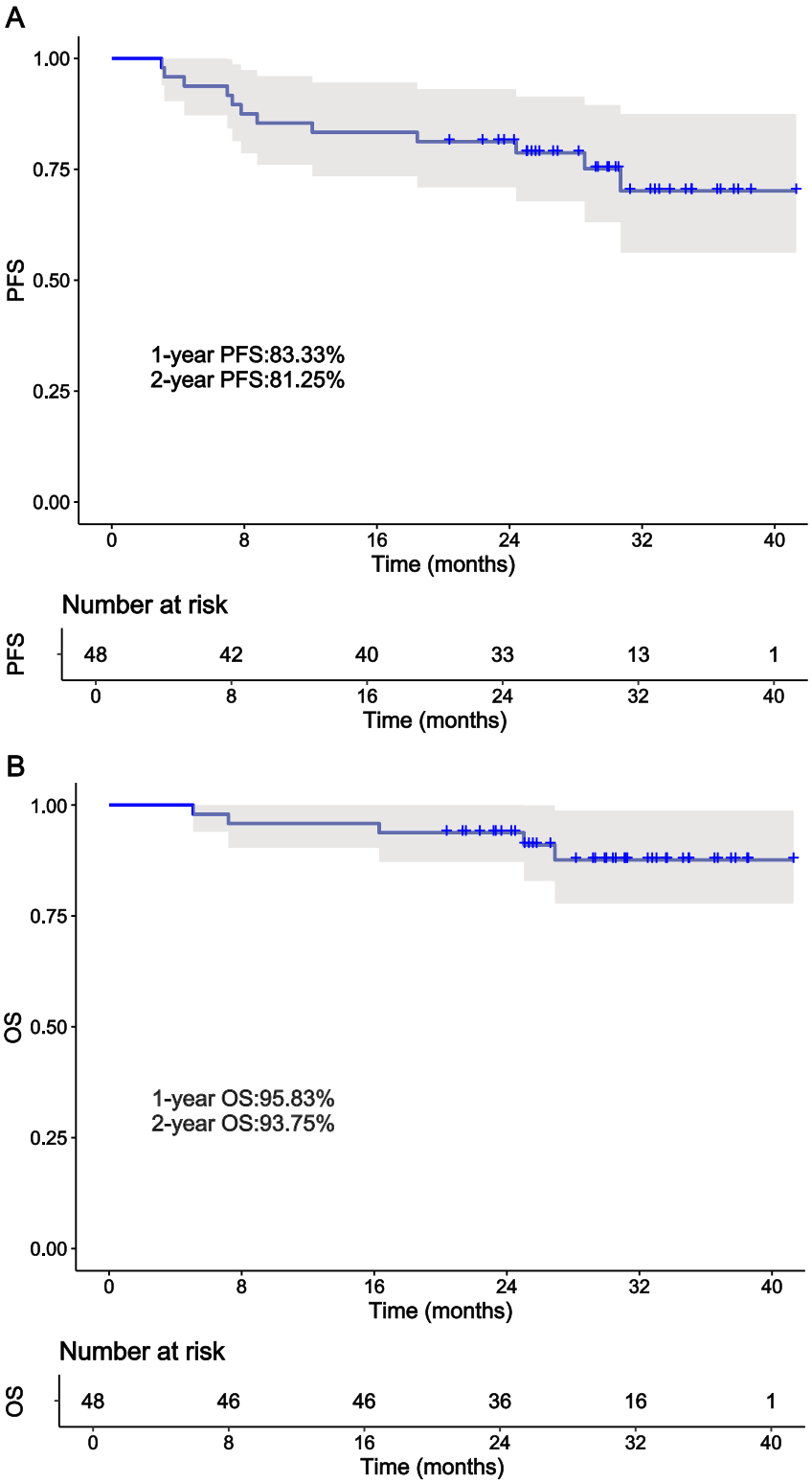


**FIGURE 2** The PFS and OS of DEL patients treated with ZR-CHOP. (A) Kaplan–Meier survival curve for PFS. (B) Kaplan–Meier survival curve for OS. DEL indicates double-expressor lymphoma; OS, overall survival; PFS, progression-free survival; ZR-CHOP, zanubrutinib in combination with R-CHOP.

poor PFS. Moreover, mutation of *NOTCH1* (HR, 7.12; 95% CI, 1.16–43.36; $p = .033$), *CDKN2A* (HR, 10.62; 95% CI, 1.76–63.98; $p = .01$), *IRF8* (HR, 15.49; 95% CI, 1.4–170.89; $p = .025$), and *BCL10* (HR, 6.98; 95% CI, 1.15–42.06; $p = .034$) were found to be associated with poor OS. However, no significant correlation was observed between *TP53* mutations and either PFS (HR, 0.24; 95% CI, 0.03–1.97; $p = .18$) or

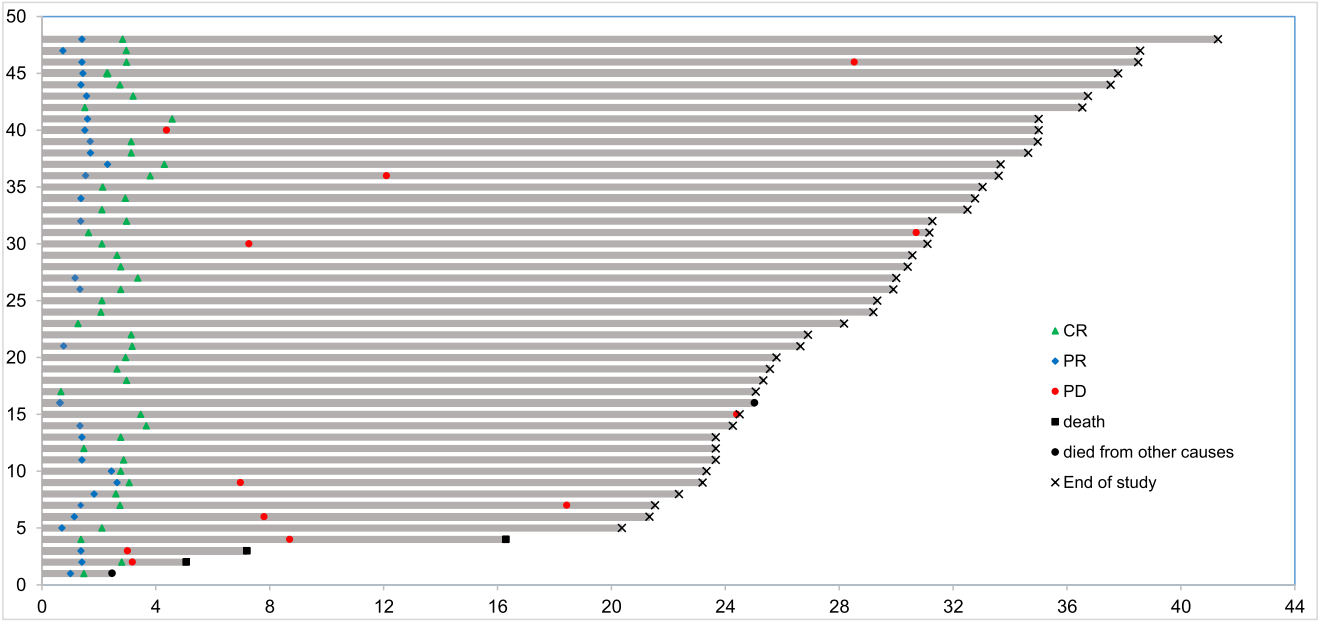


**FIGURE 3** Swimmer plot of patients enrolled in the trial. Each row represents a patient and the length of each bar represents the time from treatment initiation. CR indicates complete response; PD, progressive disease; PR, partial response.

**TABLE 2** Univariate and multivariate analysis for PFS.

| | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| **Variable** | **HR (95% CI)** | ***p*** | **HR (95% CI)** | ***p*** |
| Gender | 1.698 (0.544–5.298) | .362 | | |
| Age (<60; ≥60), years | 0.832 (0.263–2.636) | .755 | | |
| No. of extranodal involvement (0–1; ≥2) | 6.519 (1.747–24.325) | .005 | 5.6 (0.807–39.179) | .081 |
| LDH (normal; rise) | 1.922 (0.561–6.587) | .298 | | |
| Ann Arbor stage (II; III–IV) | 10.734 (1.385–83.192) | .023 | 2.66 (0.21–33.73) | .450 |
| IPI risk group (0–2; 3–5) | 2.88 (0.864–9.6) | .085 | | |
| Cell of origin (Hans) non-GCB; GCB | 0.696 (0.09–5.4) | .729 | | |
| *BCL-6* rearrangement | 0.289 (0.084–0.997) | .049 | 0.247 (0.068–0.9) | .034 |
| *TP53* mutation | 0.24 (0.03–1.97) | .18 | | |
| ECOG | 1.308 (0.541–3.163) | .551 | | |

Abbreviations: CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; GCB, germinal center B-cell type; HR, hazard ratio; IPI, International Prognostic Index; LDH, lactate dehydrogenase; non-GCB, nongerminal center B-cell type; PFS, progression-free survival.

OS (HR, 0.519; 95% CI, 0.057–4.713; $p = .56$) in the 33 patients who underwent *TP53* gene testing.

Because only 33 cases underwent NGS testing, the 15 patients did not undergo NGS testing due to insufficient material, and we must exclude the differences between patients who did not undergo testing and those who did. First, there was no significant difference in the sources of the two groups, seven of the 15 patients came from Shandong Cancer Hospital, whereas the other eight were from other research centers. Second, a demographic analysis showed that the overall proportions of gender, age (<60; ≥60), LDH (normal; elevated), Ann Arbor stage (II; III–IV), and IPI risk group (0–2; 3–5) were similar between the two groups (Table S1). However, regarding the number of extranodal involvement, patients who did not undergo NGS had a lower percentage of those with two or more extranodal sites (42.4% vs. 26.7%). Additionally, 100% of the patients without NGS were identified as non-GCB. In terms of survival analysis, there were no statistically significant differences in PFS ($p = .792$) and OS ($p = .122$) between NGS-tested and non-NGS-tested patients (Figures S1).

Additionally, 44 patients underwent FISH testing for *BCL-2*, *BCL-6*, and *MYC* genes. We found a total of eight patients with *BCL-6* rearrangement. Among the 12 patients with PD, four patients were *BCL-6* rearrangement. Among the three patients who died due to the disease, one patient had *BCL-6* rearrangement, one had both *BCL-6*

**TABLE 3** Univariate and multivariate analysis for OS.

| | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| **Variable** | **HR (95% CI)** | ***p*** | **HR (95% CI)** | ***p*** |
| Gender | 0.356 (0.04–3.187) | .356 | | |
| Age (<60; ≥60), years | 5.276 (0.589–47.254) | .137 | | |
| No. of extranodal involvement (0–1; ≥2) | 9.097 (1.00–82.694) | .05 | 15.12 (1.07–213.65) | .044 |
| LDH (normal; rise) | 77.779 (0.056–108516) | .239 | | |
| Ann Arbor stage (II; III–IV) | 3.8 (0.431–34.665) | .227 | | |
| IPI risk group (0–2; 3–5) | 6.1 (0.691–55.426) | .103 | | |
| Cell of origin (Hans) non-GCB; GCB | 1.678 (0.185–15.21) | .646 | | |
| *BCL-6* rearrangement | 0.113 (0.019–0.686) | .018 | 0.057 (0.006–0.591) | .016 |
| *TP53* mutation | 0.519 (0.057–4.713) | .56 | | |
| ECOG | 2.25 (0.711–7.1) | .167 | | |

Abbreviations: CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; GCB, germinal center B-cell type; HR, hazard ratio; IPI, International Prognostic Index; LDH, lactate dehydrogenase; non-GCB, nongerminal center B-cell type; PFS, progression-free survival.

rearrangement and *TP53* mutation, and another had *TP53* mutation. Univariate and multivariate analyses showed that *BCL-6* rearrangement was an adverse prognostic factor for PFS and OS in patients with DEL ($p < .05$).

## Toxicity and safety

Treatment was well tolerated with no dose reductions or interruptions, and the most common grade ≥3 adverse events were neutropenia (18; 37.5%), pulmonary infection (11; 22.9%), febrile neutropenia (three; 6.3%), anemia (four; 8.3%), and thrombocytopenia (four; 8.3%). All the details of the AEs are summarized in Table 4.

# DISCUSSION

DEL was characterized by the overexpression of MYC and BCL-2 proteins, which is unrelated to gene rearrangements. It primarily originates from nongerminal center B-cell type (non-GCB) and is highly aggressive. Patients with DEL have a poor prognosis with the standard R-CHOP treatment regimen.

Until recently, little prospective study has been made to improve the prognosis of patients with DEL. Several retrospective studies have found no benefit with the intensified DA-EPOCH-R regimen.[4,5] Similarly, in the context of chemotherapy followed by autologous transplantation, the SWOG 9704 study and the DLCL04 study showed a tendency to improve the prognosis of DEL patients, but the small sample size remains a limitation.[15,16]

Some other studies explore the effects of the R-CHOP regimen combined with other treatments. The POLARIX study aimed to compare the 2-year PFS of R-CHOP with that of Pola-R-CHP in patients with intermediate-risk or high-risk DLBCL (IPI ≥2) who had not received prior therapy. Subgroup analyses of DEL patients showed improved PFS for patients treated with Pola-R-CHP regimen than those with R-CHOP,[17] but no confirmation study has been made and the cost of Pola imposes a financial burden on patients. In a randomized, double-blind, placebo-controlled, multicenter phase 3 clinical trial (DEB study), the efficacy and safety of chidamide, a histone deacetylase inhibitor that promotes apoptosis in tumor cells by directly downregulating MYC and BCL2 proteins and upregulates CD20 expression to increase tumor cell sensitivity to rituximab, combined with R-CHOP were compared to R-CHOP in treatment-naive patients with DEL patients.[18,19] Interim results indicated that chidamide combined with R-CHOP significantly improved the CRR in DEL patients compared to the standard R-CHOP regimen (73.0% vs. 61.8%). Additionally, 24-month event-free survival also showed a clear trend of benefit (58.9% vs. 46.2%). Chidamide combined with R-CHOP is approved as a new regimen for DEL in China, however, the 2-year EFS is still unsatisfactory.[20] The present study using ZR-CHOP achieved CRR of 83.3% and 2-year PFS of 81.25%, which is relatively higher than any other studies reported up to now.

Another investigation is a randomized phase 2/3 study on treating previously untreated DEL patients with R-CHOP with or without the BCL-2 inhibitor venetoclax. The results showed no statistical difference in estimated 12-month PFS and OS between the two groups (PFS: HR, 0.98; 95% CI, 0.48–2.01; $p = .95$ and OS: HR, 1.27; 95% CI, 0.57–2.79; $p = .56$). The addition of venetoclax to R-CHOP led to increased toxicity (Alliance A051701).[21]

The Phoenix study found that IR-CHOP improved EFS and OS in DEL subgroup patients younger than 60 years. However, no improvement was observed in patients 60 years older, due to the increased AEs and consequently insufficient use of both ibrutinib and chemotherapy.[22]

Zanubrutinib is a next-generation small-molecule BTKi with less toxicity due to less off-target effects on EGFR, TEC, ITK, and other kinases.[23] Currently, it has been approved for the treatment of

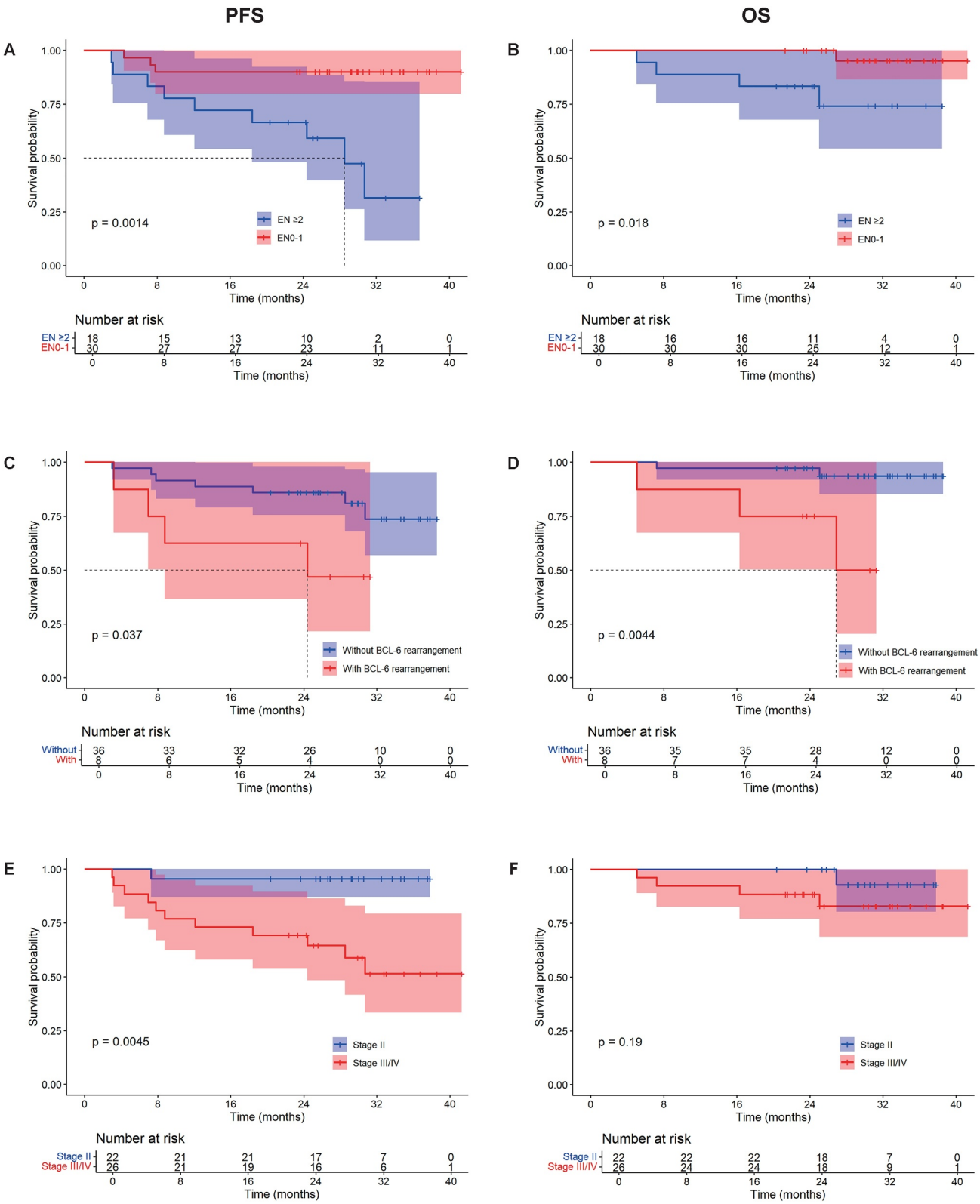


**FIGURE 4** PFS and OS of DEL patients received ZR-CHOP. Prognostic effector of EN (A and B), *BCL-6* gene rearrangement (C and D), and Ann Arbor stage (E and F) for PFS and OS. Abbreviations: DEL indicates double-expressor lymphoma; EN, number of extranodal involvements; OS, overall survival; PFS, progression-free survival; ZR-CHOP, zanubrutinib in combination with R-CHOP.

mantle cell lymphoma, adult chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), and Waldenström macroglobulinemia (WM). Consequently, an increasing number of studies are investigating the efficacy of ZR-CHOP for various forms of malignant lymphoma. For instance, a study on the ZR-CHOP regimen for newly diagnosed non-GCB DLBCL patients with multiple extranodal involvement reported an ORR of 91.7%, a CRR of 79.2%, an estimated 2-year PFS of 83.1%, and a 2-year OS of 87.1%.[24] Additionally,

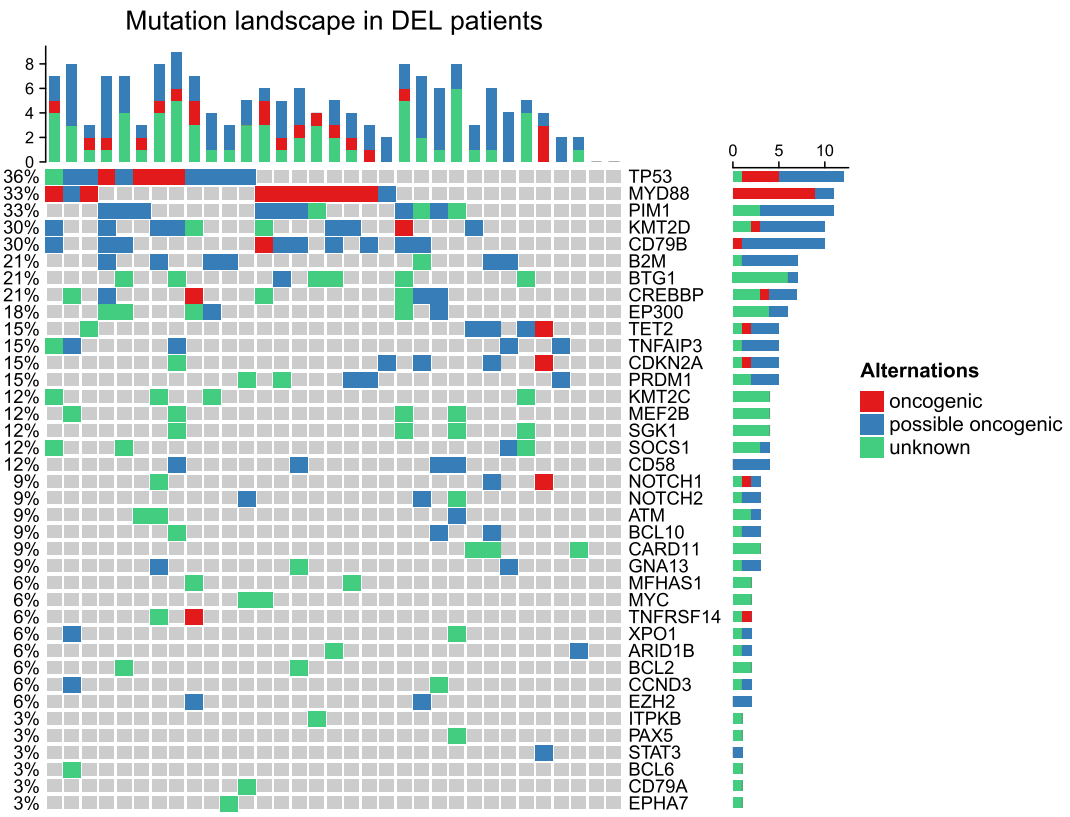


**FIGURE 5** Mutation plot for 33 double-expressor lymphoma cases. Red represents oncogenic, blue represents possible oncogenic, and green represents unknown.

**TABLE 4** AEs in DEL patients receiving ZR-CHOP.

| | Patients with events, No. (%) | |
|---|---|---|
| **AEs** | **All grades** | **Grade 3–4** |
| Fever | 12 (25.0) | 0 |
| Febrile neutropenia | 3 (6.3) | 3 (6.3) |
| Gastritis | 1 (2.1) | 0 |
| GERD | 6 (12.5) | 0 |
| Dyspepsia | 9 (18.8) | 0 |
| Oral mucositis | 9 (18.8) | 0 |
| Edema of the limbs | 2 (4.2) | 0 |
| Fatigue | 19 (39.6) | 0 |
| Pulmonary infection | 16 (33.3) | 11 (22.9) |
| Upper respiratory tract infection | 2 (4.2) | 0 |
| Skin Infection | 2 (4.2) | 0 |
| Increased alanine aminotransferase | 11 (22.9) | 0 |
| Increased aspartate transferase | 7 (14.6) | 0 |
| Anemia | 25 (52.1) | 4 (8.3) |
| Neutropenia | 34 (70.8) | 18 (37.5) |
| Thrombocytopenia | 11 (22.9) | 4 (8.3) |

Abbreviations: AEs, adverse events; DEL, double-expressor lymphoma; GERD, gastroesophageal reflux; ZR-CHOP, zanubrutinib in combination with R-CHOP.

a study involving untreated DLBCL patients with specific gene expression demonstrated an ORR of 90%, a CRR of 76%, a 2-year PFS of 74%, a 2-year OS of 87%, and a complete metabolic response (CMR) of 82% (MCD type).[25] The research on intravascular large B-cell lymphoma (IVBCL) reported a CRR of 12 of 13. All these studies achieved high response rates and showed overall favorable safety profiles, with significant hematological toxicity primarily manifesting as neutropenia (66.7%; 5 of 55; 15 of 23). Notably, in the study involving newly diagnosed non-GCB DLBCL patients with multiple extranodal involvements, two patients experienced atrial fibrillation, whereas no occurrences were reported in other studies.[26]

However, no prospective study has been reported on DEL. That is why this study was initiated. As expected, ZR-CHOP got a CRR of 83.3% and a 2-year PFS of 81.25% after almost 30 months of follow-up. Our results confirmed that no significant differences in PFS (HR, 0.832; 95% CI,0.263-2.636; $p = 0.755$) and OS (HR, 5.276; 95% CI, 0.589–47.254; $p = .137$) across age groups. Compared to the ibrutinib group in the Phoenix study, zanubrutinib demonstrated better safety (all-grade adverse events: 77.55% vs. 100%; grade ≥3 adverse events: 47.91% vs. 89.9%) and no patients discontinued due to adverse events.

Nevertheless, phase 3 trials comparing zanubrutinib monotherapy to ibrutinib monotherapy in relapsed/refractory CLL and WM revealed that neutropenia occurred more frequently in the zanubrutinib arms.[27] Notably, the incidence of ≥3 grade neutropenia in our study was significantly lower than that observed in the

PHOENIX study. This phenomenon may be influenced by various factors.[22] First, differences in patient baseline characteristics may play an important role. In the PHOENIX study, the overall incidence of bone marrow involvement in the IR-CHOP group was 11.9%, with 12.5% in patients under 60 years old[22]; in our study, the overall incidence of bone marrow involvement was 8.3% (four of 48), which may contribute to the lower incidence of neutropenia in our study. Second, in the ALPINE study, although neutropenia was more common in the zanubrutinib group (16%) compared to the ibrutinib group (13.9%),[27] the analysis of the Chinese subgroup in ALPINE showed that the incidence of neutropenia ≥3 grade was higher in the ibrutinib group (30.2%) than in the zanubrutinib group (24.4%).[28] Third, age may also be a contributing factor, in the PHOENIX study, 62.8% of patients in the IR-CHOP group were ≥60 years old, whereas in our study, the proportion of patients ≥60 years old was 45.8%. Additionally, compared to the ALPINE and PHOENIX studies, our study has a smaller patient population, which presents certain limitations.[22,27] Finally, although the study protocol did not mandate preventive measures for neutropenia, researchers at each study center used granulocyte colony-stimulating factor (G-CSF) for proactive secondary prevention at their discretion. This may lead to a lower incidence of neutropenia in our study.

The mechanism of BTKi on DEL remains unclear. It is supposed that the B-cell receptor (BCR) and nuclear factor–κB (NF-κB) signaling transduction are involved in the pathogenesis of DLBCL.[29,30] The activated NF-κB promotes the expression of BCL-2 and MYC. BTKi blocked the BCR and NF-κB pathway, reducing the expression of the two proteins and overcoming their influence.

Based on the FISH results of 44 patients, our study found that the presence of *BCL-6* rearrangement was a poor prognostic factor for DEL patients, both in univariate and multivariate analyses. *BCL-6* rearrangement patients are often associated with high expression of the BCL-6 protein. In previous studies, the expression level of BCL-6 is significantly correlated with the OS and PFS of DLBCL patients ($p < .05$).[31,32] Typically, positive expression of BCL-6 is associated with better OS ($p = .02$).[33] Studies show that BCL-6–/MYC+ expressing patients have poorer PFS than those expressing BCL-6+/MYC+ ($p < .05$).[34] However, in the DLBCL patients with high expression of MYC and BCL-2 proteins, additional *BCL-6* rearrangement causes poor outcomes. The detailed mechanisms underlying this discrepancy require further study.

*TP53* is a critical oncogene, with a mutation rate as high as 21% in patients with primary DLBCL. Patients with *TP53* expression greater than 50% are not responsive to traditional R-CHOP regimens.[35] *TP53* mutation is an independent marker of poor prognosis in DLBCL patients.[36,37] Various previous treatments have failed to alter the adverse prognostic effects of *TP53* mutations, even with CAR-T–cell therapy.[38] In our trial, NGS was performed on 33 patients, 12 of whom had *TP53* mutations. The patients with *TP53* mutations have relatively poor outcomes versus those with wild-type *TP53*, but the difference was not significant ($p > .05$). This could be attributed to the small sample size.

Among the patients who refractory or relapsed by the follow-up date, most had three or more mutated genes, suggesting that the overall accumulation of various mutated genes may be a factor contributing to poorer prognosis.

Our study has several limitations compared to other studies. First, the sample size is small, and a larger sample size study is required. Second, our study is a single-arm phase 2 trial, the comparison above mentioned is indirect and based on different studies. Despite these disadvantages, ZR-CHOP demonstrated an excellent response rate and 2-year PFS and OS in DEL patients with stage II or late disease. Additionally, the regimen is well tolerated, especially for patients ≥60 years old, who did not benefit from IR-CHOP in the Phoenix study. These findings encourage us to initiate the multicenter, phase 3 clinical study, and the underlying mechanisms of BTKi on DEL are under investigation, as well as the prognostic factors.

## AUTHOR CONTRIBUTIONS

**Xia Yin**: Data curation; validation; writing–original draft; investigation; visualization; formal analysis; and writing–review and editing. **Qiang He**: Conceptualization; data curation; writing–review and editing; funding acquisition; supervision; and methodology. **Dan Liu**: Data curation; formal analysis; methodology; and writing–review and editing. **Linna Xie**: Data curation and writing–review and editing. **Hui Wang**: Data curation and writing–review and editing. **Chunyan Chen**: Data curation and writing–review and editing. **Chuanli Zhao**: Data curation and writing–review and editing. **Ningning Shan**: Data curation and writing–review and editing. **Shanshan Shi**: Data curation and writing–review and editing. **Haichen Wei**: Data curation and writing–review and editing. **Ji Ma**: Data curation and writing–review and editing. **Ke Lu**: Data curation and writing–review and editing. **Liang Wang**: Data curation and writing–review and editing. **Yan Wang**: Data curation and writing–review and editing. **Lijie Xing**: Data curation; conceptualization; methodology; writing–review and editing; visualization; and supervision. **Zengjun Li**: Resources; project administration; writing–review and editing; funding acquisition; methodology; conceptualization; and supervision.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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