Original Article
Chidamide represses MYC expression and might improve survival for patients with double expressor lymphoma

Pengpeng Liu[1*], Xiaohang Hang[2*], Jianjun Li[1*], Lei Zhao[2], Weiping Liu[3], Jie Ji[1], Yu Wu[1], Xudong Wan[2], Xiao Shuai[1], Yong Guo[1], Bing Xiang[1], Jiazhuo Liu[1], Jie Huang[1], Zhigang Liu[1], Li Hou[1], Chong Chen[2], Yu Liu[2], Ting Liu[1]

[1]Department of Hematology, Institute of Hematology, West China Hospital, Sichuan University, Chengdu 610041, Sichuan, China; [2]State Key Laboratory of Biotherapy and Cancer Center, West China Hospital, Sichuan University, Chengdu 610041, Sichuan, China; [3]Department of Pathology, West China Hospital, Sichuan University, Chengdu 610041, Sichuan, China. *Equal contributors.

Abstract: Double expressor lymphoma (DEL), characterized by high expressions of both MYC and BCL-2, displays poor prognosis after current therapies. The HDAC inhibitor chidamide has been approved for treatment of T cell lymphoma, but its efficacy on B cell lymphoma is unclear. Here, by combining inhibition screening and transcriptomic analyses, we found that the sensitivity of B lymphoma cells to chidamide was positively correlated with the expression levels of MYC. Chidamide treatment reduced MYC protein levels and repressed MYC pathway in B lymphoma cells with high MYC expressions. Ectopic expression of MYC in chidamide-insensitive B lymphoma cells increased their response to chidamide. Thus, we proposed that adding chidamide into R-CHOP (CR-CHOP) might be effective for DEL, and retrospectively analyzed 185 DEL patients treated in West China Hospital. 80% of patients showed response to CR-CHOP treatment. In the median follow-up of 42 months, CR-CHOP significantly improve the survival for DEL patients with R-IPI ≤2. Totally 35 patients underwent autologous stem cell transplantation (ASCT) in remission and demonstrated a trend for better survival. Combining CR-CHOP with ASCT resulted in the most superior PFS and OS above all. For response patients, CR-CHOP reduced relapse with better PFS than R-CHOP-like regimens with or without ASCT. Taken together, our data indicated that chidamide repressed the MYC pathway in B lymphoma and is potentially efficacious to treat DEL.

Keywords: Chidamide, double expressor lymphoma, epigenetics, HDAC inhibitor, MYC

Introduction

Diffuse large B cell lymphoma (DLBCL) is a highly heterogeneous disease with various clinical-pathological subtypes and molecular subsets, leading to different clinical outcomes [1, 2]. MYC is a transcription factor that has been recognized as an oncogene in many different kinds of tumors. Overexpression of *MYC* has been identified in one-third of DLBCL, suggesting an important role of *MYC* in the development of DLBCL [3]. Among them, co-overexpression of *MYC* and *BCL-2* identifies a specific subtype of DLBCL, termed double-expressor lymphoma (DEL) [4], which demonstrated to have an inferior survival under current stan-

dard R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) treatment [5-7]. Previous research reported that the 3-year progression-free survival (PFS) and overall survival (OS) is 39% and 43% in DEL patients, compared to 75% PFS and 86% OS in the patients without *MYC/BCL-2* dual overexpression [5]. Intensive treatment like etoposide plus R-CHOP (R-EPOCH) or R plus dose-adjusted EPOCH (R-DA-EPOCH) is suggested for DEL patients. However, these intensive strategies turn out to be more toxic and didn't improve survival for DEL patients compare with R-CHOP in multiple studies [8, 9], except one retrospective article demonstrated better PFS and OS for young patients under 65 years old

[10]. Combining novel agents (venetoclax, ibrutinib, lenalidomide, and vorinostat) with R-CHOP have been investigated, but the supporting evidence from these reports are limited [11-14]. Therefore, developing more potential drugs and strategies for patients with DEL is urgent.

Histone deacetylases (HDACs), a family of enzymes that deacetylate the acetyl group from lysine, play important roles in cancer biology by regulating histone modification, chromatin opening, and gene expression. Inhibition of HDACs has shown great promise in cancer therapy [15]. Vorinostat is the first FDA (Food and Drug Administration)-approved HDAC inhibitor to treat cutaneous T cell lymphoma. After that, three other HDAC inhibitors (romidepsin, belinostat, panobinostat) were licensed or underwent important development [16]. Later, a new oral HDAC inhibitor chidamide has been approved for relapsed or refractory peripheral T cell lymphoma as a single agent in China [17]. Chidamide inhibits the activity of HDAC 1, 2, 3 and 10, resulting in cell growth arrest and apoptosis [18]. Despite its efficacy for T cell lymphomas, the effect of HDAC inhibitors in B-cell lymphomas is uncertain [17, 19-22]. Considering the high heterogeneity of B cell lymphomas, it is better to evaluate the drug effects in a more specific subtype.

In this study, we explored the potential efficacy of chidamide on B cell lymphoma through inhibition screening and molecular mechanism analysis. We found that the sensitivity of B lymphoma cells to chidamide was positively correlated with the expression levels of *MYC*. Thus, we proposed the application of adding chidamide to R-CHOP regimen (CR-CHOP) for DEL patients, and retrospectively analyzed the clinical outcomes of chidamide treatment on DEL patients.

Material and methods

Cell culture

The lymphoma cell lines used in the study included Granta-519 (RRID:CVCL_1818), Namalwa (RRID:CVCL_0067), and Raji (RRID:CVCL_0511) purchased from ATCC, Karpas-299 (RRID:CVCL_1324) and JeKo-1 (RRID:CVCL_1865) kindly provided by Pro. SY Yang, and CA46 (RRID:CVCL_1101), Daudi (RRID:CVCL_0008), SU-DHL-6 (RRID:CVCL_0597),

and Ramos (RRID:CVCL_0597) kindly provided by Pro. YL Zhao. The lymphoma cell lines were cultured in RPMI1640 medium (Thermo Fisher Scientific) supplemented with 10% or 20% (SU-DHL-6 and Daudi) FBS (Gibco) and 1% P/S. 293T cells were cultured in DMEM supplemented with 10% FBS and 1% P/S. 3T3 cells were cultured in DMEM medium with 10% NCS and 1% P/S. All experiments were performed with mycoplasma-free cells. All human cell lines have been authenticated using STR (or SNP) profiling within the last three years.

MYC overexpression based on retrovirus

The cDNA overexpression plasmid backbone was pMSCV-cDNA-IRES-GFP. Human MYC protein-coding sequence was obtained by PCR from the genome of Raji cells and cloned into the plasmid by BglII and EcoRI endonuclease (New England BioLabs), with empty vector as the negative control. The 293T cells were 1:2~1:2.5 passaged the day before the virus package. 20 μg plasmid, pcleo, vsvg were used to package retrovirus by calcium phosphate-DNA co-precipitation. Viruses were collected to infect Karpas299 cells 24-48 hours after transfection.

Cell growth inhibition assay

Lymphoma cells were seeded into 96-well plates at the concentration of 10^4 per well for chidamide sensitivity analyses. Chidamide was dissolved in DMSO diluted with a 2-fold gradient for lymphoma cell inhibition assay. The Cell density of each cell line was counted by flow cytometer after treated with chidamide for 48 and 72 hours.

Gene expression level detection

RNA: Lymphoma cells were collected pre- and post-treatment with chidamide. RNA was isolated with Trizol (Thermo Fisher Scientific), cDNA was synthesized with HiScript-III RT SuperMix kit (Vazyme R323-01), Fast SYBR Green Mastermix (Vazyme Q711-02/03) was used and qPCR was performed and analyzed on a StepOne Real-Time PCR System (Applied Biosystems Q3). The sequence of human *MYC* RT-PCR primer from 5' to 3' is "TTCTCTCCG-TCCTCGGATT".

Protein: Protein from lymphoma cells was prepared in laemmli buffer, separated by SDS/

PAGE electrophoresis with 5% concentration gel and 12.5% separation gel, and transferred onto PVDF membranes. Then the MYC (Proteintech, 10828-1-AP), CDK4 (Huabio ET1612-23), and Actin (Invitrogen, beta-actin-HRP, RK241732) antibodies were used to show the target protein.

Xenograft experiment

2×10^6 CA46 and KARPAS-299 cells were injected subcutaneously into both flanks of nude mice (6-week-old) to establish the lymphoma xenograft model. Treatment started when tumor became about 5 × 5 mm in surface (day 0). Chidamide was given intragastric administration every day for 16 days. Tumor volumes were monitored every 3 days, and calculated as 0.5 × a × b2 ('a' is the length and 'b' is the width).

RNA-sequencing analysis

According to the Illumina standard protocols, RNA libraries were prepared and used for sequencing. The RNA-seq data were sequenced by Illumina NovaSeq 6000 sequencing machine with 150 bp paired-end reads. The company carried out quality control (removed adapter, poly-N, and low-quality reads) on the raw data to get clean data. The contents of Q20 (>90), Q30 (>85), and GC in the clean data met the standard and could be used for downstream analysis.

The RNA-seq reads were aligned to the reference genome (hg19) by STAR [23], and Transcript was normalized by Transcript per million (TPM). DESeq2 was used for differential expression analysis, and genes with log2-fold change ≥0.5 or ≤-0.5; FDR ≤0.01 were identified as significantly differentially expressed genes [24]. GSEA used statistical approaches to identify similarities and differences between two given clusters by identifying prior-defined gene sets [25]. Differently expressed genes in pathway enrichment analysis were performed by ClusterProfiler [26]. Heatmap of differentially expressed genes of the chidamide-treated group compared with vehicle-treated group constructed by heatmap and was normalized by z-scores. The intersection genes down-regulated in the JeKo-1 chidamide-treated group and the Granta-519 chidamide-treated group, as well as the intersection with the MYC target genes, were analyzed by VennDiagram [27].

Patients

We performed a retrospective analysis of the clinical outcomes of DEL patients treated with CR-CHOP or R-CHOP-like regimen. Patients with newly diagnosed DEL between 2017 and 2020 in West China Hospital were included (ChiCTR2000041326). The diagnosis was performed according to the 2016 World Health Organization classification [28]. Cell of origin (COO) profile was determined by Hans algorithm, and DEL had been defined as MYC ≥40% and BCL-2 ≥50% in Immunohistochemistry staining. Besides, Ann Arbor stages and R-IPI (revised International Prognosis Index) [29] scores were evaluated for every patient. Patients with DHL, HIV positivity, or primary central nervous system (CNS) lymphoma were excluded. At least one cycle of standard treatment and assessment to evaluate the efficacy of the treatment should be completed. The study was approved by the ethics committee of West China Hospital (HX-2020-1200).

Treatment

CR-CHOP regimen was as given as following: Rituximab 375 mg/m² administrated intravenously on day 0, cyclophosphamide 750 mg/m², doxorubicin 50 mg/m², and vincristine 1.4 mg/m² administrated intravenously on day 1, prednisone 60 mg/m² administrated orally on days 1-5, and chidamide 20 mg administrated orally on day 1, 4, 8, and 11. The regimen was repeated every 21 days intended for 6~8 cycles. Contrast-enhanced computed tomography or positron emission tomography/computed tomography was performed to evaluate the treatment response assessed by the standardized response criteria [30]. Patients treated with R-CHOP-like regimens included R-CHOP, R-EPOCH, and R-DA-EPOCH during the same period were recruited as concurrent control.

Statistics

The final follow-up data cutoff was September 1st, 2022. The median follow-up time were 43

months for CR-CHOP cohort and 40 months for R-CHOP-like cohort, respectively. The primary endpoints were PFS measured from the therapy initiation to the date of lymphoma progression, relapse, or death, and OS measured from the therapy initiation to the date of death (or from remission time in analysis for responding patients). Secondary endpoints included response rate, and adverse events (AEs). Estimates of survival were calculated by Kaplan-Meier method and compared between subgroups using the log-rank test. The mechanism study's statistical significance was determined by a two-tailed Student's t-test of 2 independent samples. The error bar in the picture represents the standard error of the mean (SEM). Statistical analysis was performed using SPSS Software, V26.0 (IBM Corporation, Armonk, NY, USA), and GraphPad Prism software V8.3.1 (San Diego, CA, USA). For all tested hypotheses, two-tailed p values <0.05 were considered significant (*P<0.05, **P<0.01, and ***P<0.001).

Data availability

RNA-seq data used in this study can be accessed under GEO accession number GSE17-3285 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE173285). The other datasets used and/or analyzed in the study are available from the corresponding author on reasonable request.

Results

Chidamide targeted the MYC pathway in B-cell lymphoma

First, we performed an inhibition screening of B cell lymphoma cell lines on chidamide. A total of nine B cell lymphoma cell lines (CA46, Daudi, Granta-519, Namalwa, JeKo-1, Karpas-299, Raji, Ramos, and SU-DHL-6) were used. Then we calculated the half-maximal inhibitory concentration (IC50) of chidamide for each cell line by curve-fitting analysis and grouped them into chidamide-sensitive (IC50: 1.063~1.652 µM, included CA46, Daudi, JeKo-1, Raji, Namalwa, and Ramos) and chidamide-insensitive (IC50: 2.839~7.959 µM, included SU-DHL-6, Karpas-299, and Granta-519) cell lines (**Figure 1A**, Supplementary Figure 1A).

To understand the molecular mechanism of chidamide on B cell lymphoma, we performed

the high throughput RNA sequencing to analyze the transcriptome change after chidamide treatment in one chidamide-sensitive cell line, JeKo-1, and one chidamide-insensitive cell line, Granta-519. As HDAC inhibitor, chidamide profoundly altered the transcriptome of the chidamide-sensitive JeKo-1 cell line, with 3,227 significantly upregulated and 1,865 downregulated genes, among which *MYC* and many *MYC* downstream target genes were significantly down-regulated (**Figure 1B**). Of note, JeKo-1 expressed a significantly higher level of *MYC* than Granta-519 (**Figure 1C**). And consistently, 27.5% of the MYC target genes were downregulated after chidamide treatment in JeKo-1, compared to 14.5% in Granta-519. Among them, 33 out of the 200 MYC target genes were specifically downregulated in JeKo-1 by chidamide, while only 7 genes in Granta-519 (**Figure 1D** and Supplementary Figure 2A).

The results led us to explore the drug effect of chidamide on *MYC* and *MYC* pathways. Gene set enrichment analysis (GSEA) results revealed that the MYC_TARGETS pathway was significantly negatively enriched in chidamide-treated JeKo-1 cells (NES: -2.44, FDR q: 0.00) (**Figure 1E**). Moreover, the MYC-binding motif (CACGTG) was found to be significantly enriched in the promoter region of genes which is down-regulated after chidamide treatment in JeKo-1 cells (P=1e-9) (**Figure 1F**). Moreover, KEGG pathway analysis showed that the ribosome biogenesis and RNA splicing pathways, which were regulated by MYC, were significantly enriched in JeKo-1 unique down-regulated genes after chidamide treatment (Supplementary Figure 2B). Besides, the cellular apoptosis and JAK-STAT3 pathway were up-regulated in chidamide-treated JeKo-1 cells, compared to vehicle-treated cells (Supplementary Figure 2C, 2D), which might contribute to chidamide-induced cell growth repression. Together, the results suggested that chidamide could target the MYC pathway to repress B cell lymphoma cell growth.

MYC expression level positively correlated with chidamide sensitivity

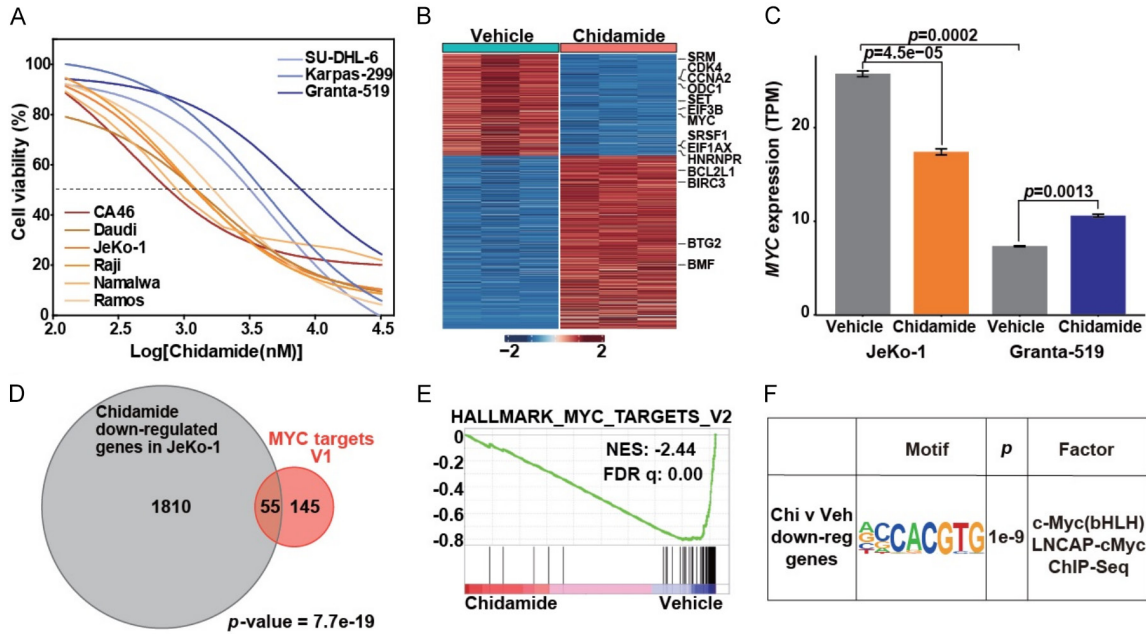Then we explored the correlation of *MYC* expression levels with chidamide sensitivity in

Figure 1. MYC pathway in B cell lymphoma cells was inhibited after chidamide treatment. A. The curve-fitting analysis of cell viability. The dotted line represents half inhibition concentration (IC50) of chidamide. B. Heatmap shows the significantly differentially expressed genes in the JeKo-1 treated chidamide or vehicle (p.adj=0.05). C. Expression levels of *MYC* in JeKo-1 and Granta-519 cells treated with vehicle and chidamide by transcriptome analysis. TPM: transcript per million. D. The Venn diagram shows the overlap of down-regulated genes in chidamide-treated JeKo-1 cells with the MYC target genes (HALLMARK MYC TARGET V1, P=7.7e-19). E. Gene set enrichment analysis (GSEA) shows that the HALLMARK MYC TARGET V2 was enriched in the vehicle-treated JeKo-1 cells. F. Binding sites of down-regulated genes in the chidamide-treated JeKo-1 cells were regulated by c-MYC.

more B cell lymphoma cells. We found that there was a significantly positive correlation between chidamide sensitivity and *MYC* mRNA level obtained from the Broad Institute Cancer Cell Line Encyclopedia (CCLE, https://portals. broadinstitute.org/ccle/) (R=-0.81, *P*=0.0082) (Supplementary Figure 1B). We confirmed the *MYC* gene expression level by RT-qPCR and observed a similar correlation (R=-0.79, *P*=0.0067 for *MYC*) (**Figure 2A**).

Then, we detected the changes of MYC and MYC-target protein levels in B cell lymphoma cells after chidamide treatment. Intriguingly, chidamide treatment significantly reduced the MYC protein levels in all chidamide-sensitive cell lines (**Figure 2B**, **2D**), while there were no significant changes in those insensitive cell lines (**Figure 2C**, **2D**). However, the mRNA levels of *MYC* were not consistently down-regulated in either chidamide-sensitive or -insensitive cells (Supplementary Figure 1C), suggesting that HDAC inhibitor chidamide targeted B-cell lymphoma more likely through suppress-

ing MYC protein directly rather than regulating at the transcriptome level. The expression of MYC-target protein, CKD4, was also down-regulated in chidamide-sensitive cell lines, but not in chidamide-insensitive cell lines (**Figure 2E**, **2F**). Meanwhile, *MYC* overexpression made chidamide-insensitive cell line, Karpas-299, more sensitive to chidamide treatment, compared to control cells infected with empty vector (**Figure 2G**). To assess activity of chidamide in vivo, we engrafted MYC-high CA46 cells and MYC-low Karpas-299 cells subcutaneously in the flanks of nude mice. Chidamide showed greater anti-tumor ability against MYC-high lymphoma cells, CA46, compared to MYC-low Karpas-299 cells (**Figure 2H**, **2I**).

These results indicated that the expression of *MYC* was related to the efficacy of chidamide, and lead us to wonder whether chidamide would be efficacious for DEL patients with overexpression of MYC and BCL-2. To examinate the effect of chidamide on MYC in DEL, we treated primary lymphoma cells from one DEL
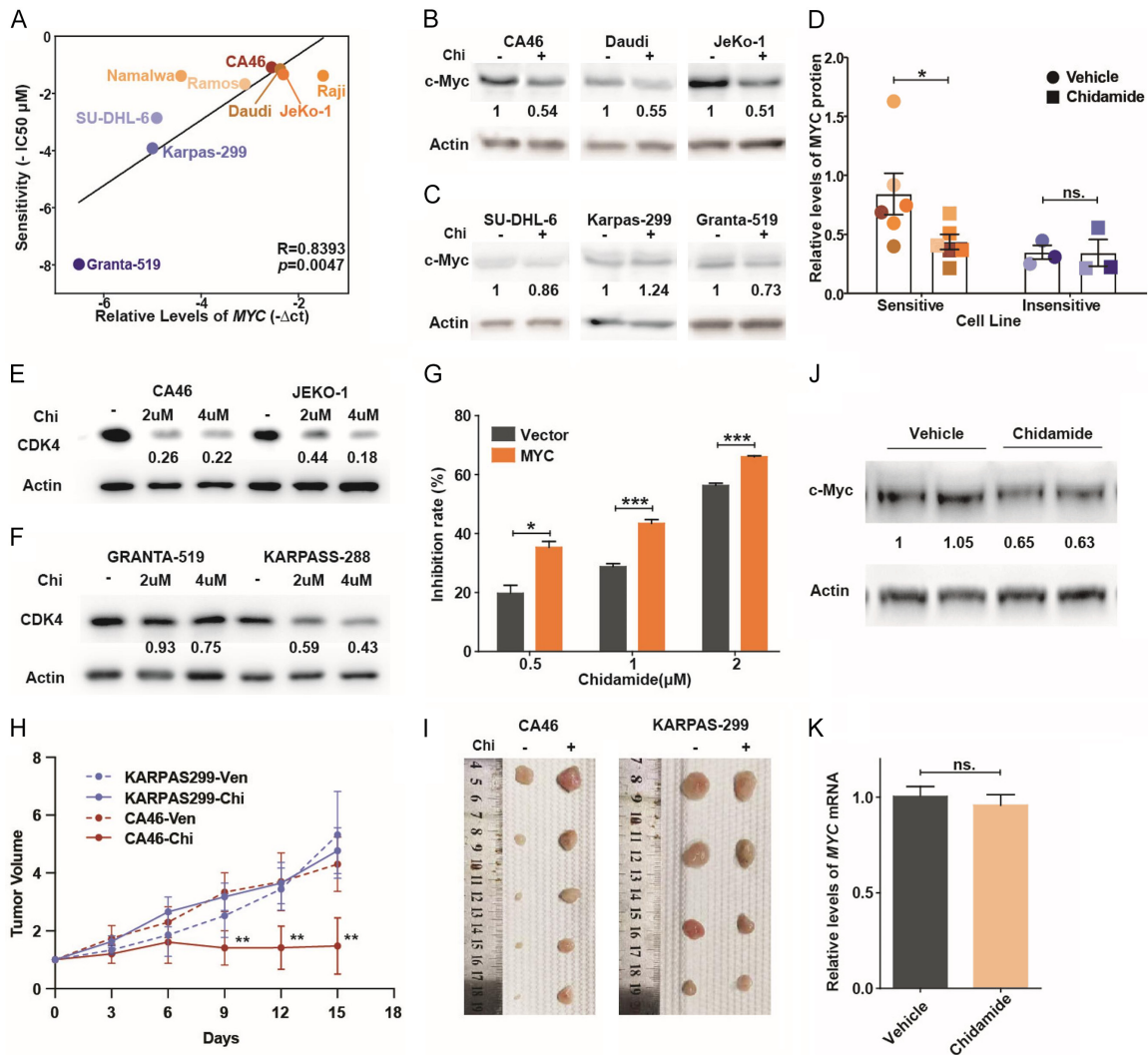
Figure 2. *MYC* expression level was positively correlated with the sensitivity of B-cell lymphoma cells to chidamide. (A) The correlation between chidamide sensitivity and *MYC* expression levels analyzed by qRT-PCR in different B-cell lymphoma cell lines (R=0.8393, P=0.0047). (B, C) Western blotting of MYC in chidamide-sensitive (B) and chidamide-insensitive (C) B-cell lymphoma cell lines treated with vehicle or 2 µM chidamide. (D) Quantitation of MYC proteins in chidamide-sensitive and chidamide-insensitive cell lines treated with vehicle or 2 µM chidamide. (E, F) Western blotting of CDK4 in chidamide-sensitive (E) and chidamide-insensitive (F) B-cell lymphoma cell lines. (G) Inhibition rate of *MYC* overexpressed Karpas-299 cells after chidamide treatment. (H, I) Tumor growth ratio curve after chidamide administration in vivo (H) and exfoliated tumor mass on day 15 (I). (J, K) The MYC protein (J) and mRNA (K) level change in lymphoma cells from a DEL patient treated with 4 µM chidamide. *P<0.05, **P<0.01, ***P<0.001 (two-tailed Student's *t*-test).

patient with chidamide in vitro. The results showed that chidamide decreased the protein level but not the mRNA level of MYC (**Figure 2J, 2K**).

Patients characteristics and response rates

Furthermore, to confirm the efficacy of chidamide in DEL. We performed a retrospective analysis comparing clinical outcomes of CR-CHOP vs R-CHOP-like regimens on patients with DEL. A total of 185 patients diagnosed with DEL between 2017 and 2020 in West China Hospital were included, of which 73 patients were treated with CR-CHOP, and 112 patients were treated with R-CHOP-like regimens during the same period as concurrent control, including R-CHOP for 90 patients, R-EPOCH for 15 patients, and R-DA-EPOCH for 7 patients. The baseline characteristics

Table 1. Baseline characteristics of DEL patients with CR-CHOP or R-CHOP-like regimens at diagnosis[a]

	CR-CHOP	R-CHOP-like	*p* value[b]
Study Population	73	112	
Sex			0.135
Male	34 (46.58%)	65 (58.04%)	
Female	39 (53.42%)	47 (41.96%)	
Age			0.756
<60 [65]	48 (65.75%)	71 (63.39%)	
≥60 [65]	25 (34.25%)	41 (36.61%)	
Mean	53.40±12.55 yr	55.79±14.80 yr	
Stage (Ann-Arbor)			0.048*
I-II	24 (32.88%)	54 (48.21%)	
III-IV	49 (67.12%)	58 (51.79%)	
Extranodal site (≥1 site)			0.290
Yes	59 (80.82%)	82 (73.21%)	
No	14 (19.18%)	30 (26.79%)	
CNS involvement	1 (1.37%)	3 (2.68%)	-
R-IPI			0.317
Very Good (0)	13 (17.81%)	22 (19.64%)	
Good (1, 2)	29 (39.73%)	52 (46.43%)	
Poor (3, 4, 5)	31 (42.47%)	38 (33.93%)	
COO			1.000
GCB	7 (9.59%)	12 (10.71%)	
Non-GCB	66 (90.41%)	94 (83.93%)	
Unclassified	0	6 (5.36%)	
Rearrangements			-
MYC	2 (2.74%)	1 (0.89%)	
BCL2	0	1 (0.89%)	
BCL6	8 (10.96%)	15 (13.39%)	
ASCT			0.007*
In CR1	18 (24.66%)	12 (10.71%)	
In CR2	3 (4.11%)	2 (1.79%)	

[a]Plus-minus values are mean ± SD. Percentages may not total 100 because of rounding. [b]The *p* values represent the test of homogeneity between patients treated with CR-CHOP and CHOP-liked regimens, calculated by Chi-Square, Wilcoxon Signed Rank or Fisher's precise test. *represents significant values (*P*<0.05). R-IPI: revised international prognostic index; COO: cell of origin; R-CHOP: rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone; CR-CHOP: Chidamide plus R-CHOP.

between the 2 groups were almost similar, except that patients in CR-CHOP group were with significantly more advanced diseases [49/73 (67.12%) vs 58/112 (51.79%) of the patients had an advanced Ann Arbor stage (III-IV stage) in the CR-CHOP and R-CHOP-like group respectively] at diagnosis (**Table 1**).

Response assessment was feasible in 178 patients (71 for CR-CHOP group, and 107 for R-CHOP-like group). The overall response rates were 80% vs 75% (P=0.392), and the complete response (CR) were 79% vs 70% (P=0.193) following CR-CHOP and R-CHOP-like regimens,

respectively. Notably, more patients with R-IPI score ≤2 had overall response (90% vs 77%, P=0.074) and CR (88% vs 73%, P=0.057) to the treatment in CR-CHOP group (**Table 2**). Seven patients were not evaluable because of adverse events (n=4, 2 died of pneumonia; 1 was due to seizure; 1 had acute exacerbation of chronic obstructive pulmonary disease) and loss to follow-up (n=3).

Most of the adverse events were manageable in CR-CHOP group, except for one patient who died from pneumonia and one patient who interrupted the regimen due to recurrent sei-

Table 2. Patient best response after induction chemotherapy

	Evaluable Patients	CR	PR	ORR	PD/SD
CR-CHOP					
Overall	71	56 (79%)	1 (1%)	57 (80%)	14 (20%)
R-IPI ≤2	42	37 (88%)	1 (2%)	38 (90%)	4 (10%)
R-IPI >2	29	19 (66%)	0	19 (66%)	10 (34%)
R-CHOP-like					
Overall	107	75 (70%)	5 (5%)	80 (75%)	27 (25%)
R-IPI ≤2	70	51 (73%)	3 (4%)	54 (77%)	16 (23%)
R-IPI >2	37	24 (65%)	2 (5%)	26 (70%)	11 (30%)

R-IPI: revised international prognostic index; CHOP: cyclophosphamide, doxorubicin, vincristine, and prednisone; CR-CHOP: Chidamide plus R-CHOP.

zure. Other ≥ grade 3 adverse events included thrombocytopenia in 2.7% patients, anemia in 8% patients, neutropenia in 21.9% patients.

Survival outcomes

During the first remission (CR1), 18 (24.66%) patients in CR-CHOP group and 12 (10.71%) patients in R-CHOP-like group underwent autologous stem cell transplantation (ASCT) as consolidative therapy. Besides, 3 patents in CR-CHOP group, and 2 patients in R-CHOP-like group received salvage ASCT in CR2 after remission from second-line chemotherapy (**Table 1**). ASCT was performed following Chi-CGB condition treatment (chidamide, cladribine, gemcitabine, and busulfan) reported in our previous study [31].

With a median follow-up of 42 months (interquartile range [IQR], 30-54 months), we explored if chidamide and ASCT affected the prognosis of DEL patients. For patients without ASCT, the overall 3-year PFS was 61.4% vs 51.2% (P=0.197) and OS was 74.7% vs 69.4% (P=0.230) following CR-CHOP and R-CHOP-like regimens (**Figure 3A**, **3B**). Intriguingly, in the subset of patients with R-IPI ≤2, CR-CHOP could significantly improve the survival (3-year PFS: 84.7% vs 59.6%, P=0.037; 3-year OS: 87.9% vs 73.5%, P=0.041) compared to R-CHOP-like regimens (**Figure 3C**, **3D**), while here is no significant difference for those with R-IPI >2 (**Figure 3E**, **3F**). Then we evaluated the efficacy of ASCT in DEL. Patients received ASCT showed a trend for better PFS and OS than others, however, without statistic signifi-

cance (Supplementary Figure 3A-F). But it is worthy to note that in the subset of R-IPI >2 group, among 13 patients, only 2 relapsed and only 1 died in ASCT cohort. Therefore, we believed that chidamide and ASCT had potential to improve the clinical outcome of DEL patients. Furthermore, patients treated with CR-CHOP regimen consolidated by ASCT presented with the most superior PFS and OS above all, and their PFS was significantly improved compared with patients treated with R-CHOP-like regimens and ASCT (3-year PFS: 94.7% vs 64.8%, P=0.047), although OS was not significantly affected. At the meanwhile, for patients who were not received ASCT after remission, CR-CHOP also reduced the chance of relapse compared to R-like-regimen (3-year PFS: 87.6% vs 72.2%, P=0.030) (**Figure 4A**, **4B**).

Discussion

In this study, we found that the sensitivity of B lymphoma cells to HDAC inhibitor chidamide was positively correlated with the expression levels of *MYC*, which led us to apply the drug into the treatment of DEL, combining with standard R-CHOP (CR-CHOP regimen). CR-CHOP turned out to be efficacious to improve the survival of DEL patients with R-IPI ≤2. Meanwhile, we suggested a treatment strategy consisting of the integration of CR-CHOP and upfront ASCT with Chi-CGB condition for DEL.

Unlike other B cell lymphomas, *MYC* and *BCL-2* overexpressed DEL, representing 20-30% cases of DLBCL, is associated with poor outcomes after current standard R-CHOP treatment, even in patients with low R-IPI score [5-7]. Existing evidence to support Intensive chemotherapy, such as R-EPOCH and R-DA-EPOCH is limited, and the benefit is restricted to young patients [10]. Thus, DEL is an urgent clinical problem and new treatment strategies are required. Novel drugs including immunomodulators like lenalidomide and small molecular inhibitors targeting BCL-2 were added into the standard R-CHOP or R-EPOCH regimen for the treatment of DEL and tested in clinical trials [11, 13, 32]. However, most of the studies were not designed specifically for DEL, so
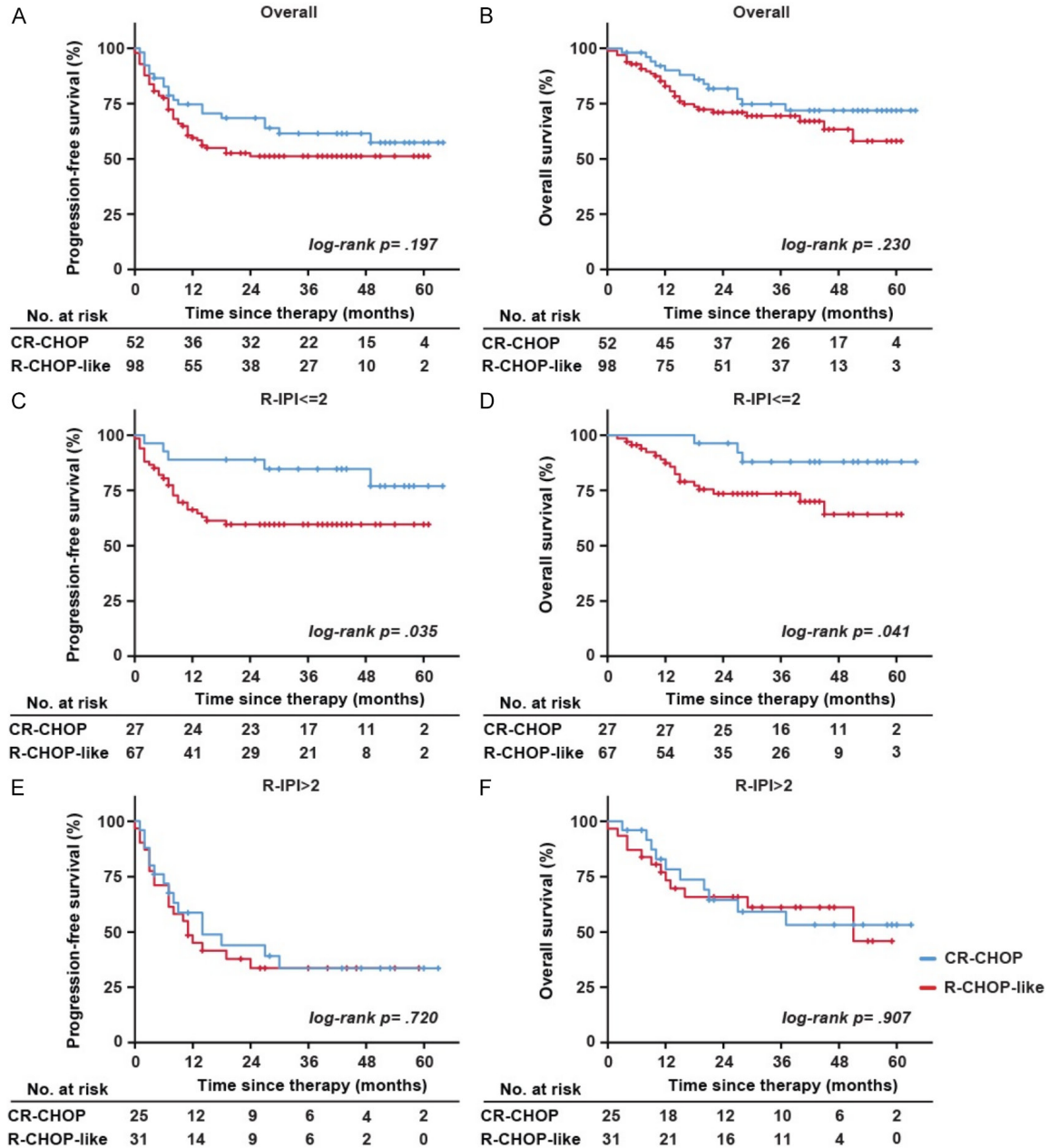
Figure 3. CR-CHOP improved survival of DEL patients with R-IPI ≤2 without transplantation. Progression-free survival (A, C, E) and overall survival (B, D, F) for all DEL patients (A, B), patients with R-IPI ≤2 (C, D) and patient with R-IPI >2 (E, F), without transplantation. R-IPI: revised international prognostic index; R-CHOP: rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone; CR-CHOP: chidamide plus R-CHOP.

they only enrolled a very small number of DEL patients. So far, the treatment of DEL is still very challenging. Epigenetic dysregulation is a hallmark of cancer. Among them, dysregulation of HDACs plays a critical role in tumorigenesis, including B cell lymphoma. Cytotoxic effects of HDAC inhibitors on B cell lymphoma have been proven in multiple preclinical studies [33, 34].

However, supporting evidence from clinical trials is limited, and suitable subgroups are not identified. Chidamide is a novel oral HDAC inhibitor, which has been proved by China FDA to treat peripheral T cell lymphoma in 2014 [17], In our study, we showed that DEL might fit into the indication of chidamide, as the drug sensitivity is positively correlated with *MYC*
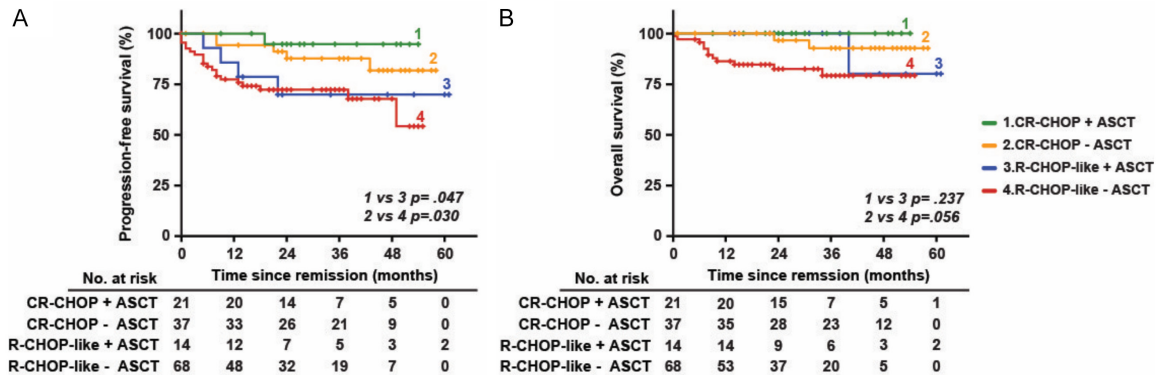
Figure 4. CR-CHOP reduced relapse for DEL patients with/without ASCT after remission. Progression-free survival (A) and overall survival (B) for DEL patient after remission. ASCT: Autologous stem cell transplantation.

expression level. 80% of patients with DEL responded to the treatment after 6 cycle of CR-CHOP regimen. Our results are comparable with a recent study in which all 12 patients with DEL reached CR after CR-CHOP treatment [22]. Also, in our study, CR-CHOP significantly improve the survival of patients with R-IPI ≤2. Moreover, a multicenter phase III clinical trial of tucidinostat (chidamide) combined with R-CHOP in patients with newly diagnosed DEL (NCT04231448) is ongoing in China.

The application of hematopoietic cell transplantation for DEL is controversial. Some studies have demonstrated poor outcomes after autologous or allogeneic transplantation for DEL [35, 36]. However, these studies mainly recruited patients with refractory or relapsed disease and at least 2 lines of chemotherapy were applied before transplantation. Upfront ASCT as consolidation is suggested for aggressive lymphoma to reduce relapse and improve survival [37]. Some studies evaluated the efficacy of upfront ASCT in DEL and survival advantage was observed [38-40], suggesting the potential of ASCT as consolidation for DEL. In our study, we also observed a trend for better survival and less relapse after ASCT. So we suggest to bring the ASCT forwards before relapse, and propose a new treatment strategy for DEL consisting of CR-CHOP followed by ASCT consolidation. We also included chidamide into the condition treatment for ASCT which showed favorable results for DEL in our previous studies [31].

Mechanically, our study has shown that HDAC inhibition could reduce protein levels of MYC in DEL lymphoma cells. Very interestingly, the mRNA level was not significantly affected after treatment. Direct regulation of MYC by HDAC in protein level has been observed in other studies when pan or Class I HDAC inhibitors were used to treat other types of cancer [41-44]. First, MYC protein can be directly acetylated by EP300 and CBP, which are the substrates of HDAC [42, 43]. It has been shown that, after HDAC inhibitor (vorinostat) treatment, MYC was acetylated at lysine 323, which led to the increased turnover of MYC protein in the histiocytic lymphoma cell called U-937 [42]. Moreover, HDAC2 was reported to be a cofactor of MYC in MYC overexpressed medulloblastoma [41]. The repression of HDAC2 could reduce the DNA binding activity of MYC protein and subsequently downregulated the expression of MYC downstream target genes [41]. However, seldom study investigated the relationship of MYC and HDAC in lymphoma. In a recent study, a new HDAC inhibitor CKD-581 also showed anti-cancer effects in DLBCL by down-regulating MYC [45], but more work needs to be done to explore the exact mechanism. Besides, as DEL has dual overexpression of *MYC* and *BCL-2*, we also investigated the relationship between *BCL-2* expression level and chidamide sensitivity. The results turned out to be insignificant. It is worthwhile to explore whether other MYC-driven cancer cells are sensitive to HDAC inhibition of chidamide.

Acknowledgements

Disclosure of conflict of interest

None.

Address correspondence to: Ting Liu, Department of Hematology, Institute of Hematology, West China Hospital, Sichuan University, No. 37 Guo Xue Xiang Street, Chengdu 610041, Sichuan, China. E-mail: liuting@scu.edu.cn; Yu Liu, State Key Laboratory of Biotherapy and Cancer Center, West China Hospital, Sichuan University, No. 37 Guo Xue Xiang Street, Chengdu 610041, Sichuan, China. E-mail: yuliuscu@scu.edu.cn

References

[1] Jiang M, Bennani NN and Feldman AL. Lymphoma classification update: B-cell non-Hodgkin lymphomas. Expert Rev Hematol 2017; 10: 405-415.

[2] Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, Rosenwald A, Boldrick JC, Sabet H, Tran T, Yu X, Powell JI, Yang L, Marti GE, Moore T, Hudson J Jr, Lu L, Lewis DB, Tibshirani R, Sherlock G, Chan WC, Greiner TC, Weisenburger DD, Armitage JO, Warnke R, Levy R, Wilson W, Grever MR, Byrd JC, Botstein D, Brown PO and Staudt LM. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 2000; 403: 503-511.

[3] Friedberg JW. How I treat double-hit lymphoma. Blood 2017; 130: 590-596.

[4] Riedell PA and Smith SM. Double hit and double expressors in lymphoma: definition and treatment. Cancer 2018; 124: 4622-4632.

[5] Green TM, Young KH, Visco C, Xu-Monette ZY, Orazi A, Go RS, Nielsen O, Gadeberg OV, Mourits-Andersen T, Frederiksen M, Pedersen LM and Møller MB. 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.

[6] Hu S, Xu-Monette ZY, Tzankov A, Green T, Wu L, Balasubramanyam A, Liu WM, Visco C, Li Y, Miranda RN, Montes-Moreno S, Dybkaer K, Chiu A, Orazi A, Zu Y, Bhagat G, Richards KL, Hsi ED, Choi WW, Zhao X, van Krieken JH, Huang Q, Huh J, Ai W, Ponzoni M, Ferreri AJ, Zhou F, Slack GW, Gascoyne RD, Tu M, Variakojis D, Chen W, Go RS, Piris MA, Møller MB, Medeiros LJ and Young KH. 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; quiz 4250.

[7] Staiger AM, Ziepert M, Horn H, Scott DW, Barth TFE, Bernd HW, Feller AC, Klapper W, Szczepanowski M, Hummel M, Stein H, Lenze D, Hansmann ML, Hartmann S, Möller P, Cogliatti S, Lenz G, Trümper L, Löffler M, Schmitz N, Pfreundschuh M, Rosenwald A and Ott G; German High-Grade Lymphoma Study Group. Clinical impact of the cell-of-origin classification and the MYC/ BCL2 dual expresser status in diffuse large B-cell lymphoma treated within prospective clinical trials of the german high-grade non-Hodgkin's lymphoma study group. J Clin Oncol 2017; 35: 2515-2526.

[8] Bartlett NL, Wilson WH, Jung SH, Hsi ED, Maurer MJ, Pederson LD, Polley MC, Pitcher BN, Cheson BD, Kahl BS, Friedberg JW, Staudt LM, Wagner-Johnston ND, Blum KA, Abramson JS, Reddy NM, Winter JN, Chang JE, Gopal AK, Chadburn A, Mathew S, Fisher RI, Richards KL, Schöder H, Zelenetz AD and Leonard JP. Dose-adjusted EPOCH-R compared with R-CHOP as frontline therapy for diffuse large B-cell lymphoma: clinical outcomes of the phase III intergroup trial alliance/CALGB 50303. J Clin Oncol 2019; 37: 1790-1799.

[9] Zhang XY, Liang JH, Wang L, Zhu HY, Wu W, Cao L, Fan L, Li JY and Xu W. DA-EPOCH-R improves the outcome over that of R-CHOP regimen for DLBCL patients below 60 years, GCB phenotype, and those with high-risk IPI, but not for double expressor lymphoma. J Cancer Res Clin Oncol 2019; 145: 117-127.

[10] Dodero A, Guidetti A, Tucci A, Barretta F, Novo M, Devizzi L, Re A, Passi A, Pellegrinelli A, Pruneri G, Miceli R, Testi A, Pennisi M, Di Chio MC, Matteucci P, Carniti C, Facchetti F, Rossi G and Corradini P. Dose-adjusted EPOCH plus rituximab improves the clinical outcome of young patients affected by double expressor diffuse large B-cell lymphoma. Leukemia 2019; 33: 1047-1051.

[11] Zelenetz AD, Salles G, Mason KD, Casulo C, Le Gouill S, Sehn LH, Tilly H, Cartron G, Chamuleau MED, Goy A, Tam CS, Lugtenburg PJ, Petrich AM, Sinha A, Samineni D, Herter S, Ingalla E, Szafer-Glusman E, Klein C, Sampath D, Kornacker M, Mobasher M and Morschhauser F. Venetoclax plus R- or G-CHOP in non-Hodgkin lymphoma: results from the CAVALLI phase 1b trial. Blood 2019; 133: 1964-1976.

[12] Johnson PWM, Balasubramanian S, Hodkinson B, Shreeve SM, Sun S, Srinivasan S, Steele AJ, Vermeulen J, Sehn LH and Wilson WH. Clinical impact of ibrutinib plus R-CHOP in untreated DLBCL coexpressing BCL2 and MYC in the phase 3 PHOENIX trial. Blood Adv 2023; 7: 2008-2017.

[13] Godfrey JK, Nabhan C, Karrison T, Kline JP, Cohen KS, Bishop MR, Stadler WM, Karmali R, Venugopal P, Rapoport AP and Smith SM. Phase 1 study of lenalidomide plus dose-adjusted EPOCH-R in patients with aggressive B-cell lymphomas with deregulated MYC and BCL2. Cancer 2019; 125: 1830-1836.

[14] Persky DO, Li H, Rimsza LM, Barr PM, Popplewell LL, Bane CL, Von Gehr A, LeBlanc M, Fisher RI, Smith SM and Friedberg JW. A phase I/II trial of vorinostat (SAHA) in combination with rituximab-CHOP in patients with newly diagnosed advanced stage diffuse large B-cell lymphoma (DLBCL): SWOG S0806. Am J Hematol 2018; 93: 486-493.

[15] Li Y and Seto E. HDACs and HDAC inhibitors in cancer development and therapy. Cold Spring Harb Perspect Med 2016; 6: a026831.

[16] Moskowitz AJ and Horwitz SM. Targeting histone deacetylases in T-cell lymphoma. Leuk Lymphoma 2017; 58: 1306-1319.

[17] Lu X, Ning Z, Li Z, Cao H and Wang X. Development of chidamide for peripheral T-cell lymphoma, the first orphan drug approved in China. Intractable Rare Dis Res 2016; 5: 185-191.

[18] Gong K, Xie J, Yi H and Li W. CS055 (Chidamide/HBI-8000), a novel histone deacetylase inhibitor, induces G1 arrest, ROS-dependent apoptosis and differentiation in human leukaemia cells. Biochem J 2012; 443: 735-746.

[19] Chen R, Frankel P, Popplewell L, Siddiqi T, Ruel N, Rotter A, Thomas SH, Mott M, Nathwani N, Htut M, Nademanee A, Forman SJ and Kirschbaum M. A phase II study of vorinostat and rituximab for treatment of newly diagnosed and relapsed/refractory indolent non-Hodgkin lymphoma. Haematologica 2015; 100: 357-362.

[20] Li Q, Huang J, Ou Y, Li Y and Wu Y. Progressive diffuse large B-cell lymphoma with TP53 gene mutation treated with chidamide-based chemotherapy. Immunotherapy 2019; 11: 265-272.

[21] Guan XW, Wang HQ, Ban WW, Chang Z, Chen HZ, Jia L and Liu FT. Novel HDAC inhibitor Chidamide synergizes with Rituximab to inhibit diffuse large B-cell lymphoma tumour growth by upregulating CD20. Cell Death Dis 2020; 11: 20.

[22] Zhang MC, Fang Y, Wang L, Cheng S, Fu D, He Y, Zhao Y, Wang CF, Jiang XF, Song Q, Xu PP and Zhao WL. Clinical efficacy and molecular biomarkers in a phase II study of tucidinostat plus R-CHOP in elderly patients with newly diagnosed diffuse large B-cell lymphoma. Clin Epigenetics 2020; 12: 160.

[23] Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M and Gingeras TR. STAR: ultrafast universal RNA-seq aligner. Bioinformatics 2013; 29: 15-21.

[24] Love MI, Huber W and Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 2014; 15: 550.

[25] Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES and Mesirov JP. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 2005; 102: 15545-15550.

[26] Yu G, Wang LG, Han Y and He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. Omics 2012; 16: 284-287.

[27] Chen H and Boutros PC. VennDiagram: a package for the generation of highly-customizable venn and euler diagrams in R. BMC Bioinformatics 2011; 12: 35.

[28] Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, Advani R, Ghielmini M, Salles GA, Zelenetz AD and Jaffe ES. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood 2016; 127: 2375-2390.

[29] International Non-Hodgkin's Lymphoma Prognostic Factors Project. A predictive model for aggressive non-Hodgkin's lymphoma. N Engl J Med 1993; 329: 987-994.

[30] Cheson BD, Fisher RI, Barrington SF, Cavalli F, Schwartz LH, Zucca E and Lister TA; Alliance, Australasian Leukaemia and Lymphoma Group; Eastern Cooperative Oncology Group; European Mantle Cell Lymphoma Consortium; Italian Lymphoma Foundation; European Organisation for Research; Treatment of Cancer/Dutch Hemato-Oncology Group; Grupo Español de Médula Ósea; German High-Grade Lymphoma Study Group; German Hodgkin's Study Group; Japanese Lymphorra Study Group; Lymphoma Study Association; NCIC Clinical Trials Group; Nordic Lymphoma Study Group; Southwest Oncology Group; United Kingdom National Cancer Research Institute. Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification. J Clin Oncol 2014; 32: 3059-3068.

[31] Ji J, Liu Z, Kuang P, Dong T, Chen X, Li J, Zhang C, Liu J, Zhang L, Shen K and Liu T. A new conditioning regimen with chidamide, cladribine, gemcitabine and busulfan significantly improve the outcome of high-risk or relapsed/refractory non-Hodgkin's lymphomas. Int J Cancer 2021; 149: 2075-2082.

[32] Nabhan C, Karrison T, Kline J, Cohen K, Bishop MR, Karmali R, Rapoport AP, Venugopal P, Wade J, Fishkin P and Smith SM. Prospective phase I multi-center trial incorporating lenalidomide (LEN) into dose-adjusted EPOCH plus

rituximab (DA-EPOCH-R) in patients with double-hit (DHL) or double expressing (DEL) lymphomas: final results. Blood 2016; 128: 4191-4191.

[33] Frys S, Simons Z, Hu Q, Barth MJ, Gu JJ, Mavis C, Skitzki J, Song L, Czuczman MS and Hernandez-Ilizaliturri FJ. Entinostat, a novel histone deacetylase inhibitor is active in B-cell lymphoma and enhances the anti-tumour activity of rituximab and chemotherapy agents. Br J Haematol 2015; 169: 506-519.

[34] Huang HL, Peng CY, Lai MJ, Chen CH, Lee HY, Wang JC, Liou JP, Pan SL and Teng CM. Novel oral histone deacetylase inhibitor, MPT0E028, displays potent growth-inhibitory activity against human B-cell lymphoma in vitro and in vivo. Oncotarget 2015; 6: 4976-4991.

[35] Herrera AF, Mei M, Low L, Kim HT, Griffin GK, Song JY, Merryman RW, Bedell V, Pak C, Sun H, Paris T, Stiller T, Brown JR, Budde LE, Chan WC, Chen R, Davids MS, Freedman AS, Fisher DC, Jacobsen ED, Jacobson CA, LaCasce AS, Murata-Collins J, Nademanee AP, Palmer JM, Pihan GA, Pillai R, Popplewell L, Siddiqi T, Sohani AR, Zain J, Rosen ST, Kwak LW, Weinstock DM, Forman SJ, Weisenburger DD, Kim Y, Rodig SJ, Krishnan A and Armand P. Relapsed or refractory double-expressor and double-hit lymphomas have inferior progression-free survival after autologous stem-cell transplantation. J Clin Oncol 2017; 35: 24-31.

[36] Kawashima I, Inamoto Y, Maeshima AM, Nomoto J, Tajima K, Honda T, Shichijo T, Kawajiri A, Takemura T, Onishi A, Ito A, Tanaka T, Fuji S, Kurosawa S, Kim SW, Maruyama D, Tobinai K, Kobayashi Y and Fukuda T. Double-expressor lymphoma is associated with poor outcomes after allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant 2018; 24: 294-300.

[37] Stiff PJ, Unger JM, Cook JR, Constine LS, Couban S, Stewart DA, Shea TC, Porcu P, Winter JN, Kahl BS, Miller TP, Tubbs RR, Marcellus D, Friedberg JW, Barton KP, Mills GM, LeBlanc M, Rimsza LM, Forman SJ and Fisher RI. Autologous transplantation as consolidation for aggressive non-Hodgkin's lymphoma. N Engl J Med 2013; 369: 1681-1690.

[38] Herrera AF, Rodig SJ, Song JY, Kim Y, Griffin GK, Yang D, Nikolaenko L, Mei M, Bedell V, Dal Cin P, Pak C, Alyea EP, Budde LE, Chen R, Chen YB, Chan WC, Cutler CS, Ho VT, Koreth J, Krishnan A, Murata-Collins JL, Nikiforow S, Palmer J, Pihan GA, Pillai R, Popplewell L, Rosen ST, Siddiqi T, Sohani AR, Zain J, Kwak LW, Weisenburger DD, Weinstock DM, Soiffer RJ, Antin JH, Forman SJ, Nademanee AP and Armand P. Outcomes after allogeneic stem cell transplantation in patients with double-hit and double-expressor lymphoma. Biol Blood Marrow Transplant 2018; 24: 514-520.

[39] Kim YR, Yoon SO, Kim SJ, Cheong JW, Chung H, Lee JY, Jang JE, Kim Y, Yang WI, Min YH and Kim JS. Upfront autologous hematopoietic stem cell transplantation for high-risk patients with double-expressor diffuse large B cell lymphoma. Ann Hematol 2020; 99: 2149-2157.

[40] Strüßmann T, Glatzki F, Engelhardt M, Mertelsmann R, Duyster J, Finke J and Marks R. Favourable outcomes of double-hit/double-expressor lymphoma and high-grade B-cell lymphoma, not otherwise specified after early dose-intensive treatment and up-front autologous stem cell transplantation: a single-centre retrospective experience. Br J Haematol 2022; 198: 776-779.

[41] Ecker J, Thatikonda V, Sigismondo G, Selt F, Valinciute G, Oehme I, Muller C, Buhl JL, Ridinger J, Usta D, Qin N, van Tilburg CM, Herold-Mende C, Remke M, Sahm F, Westermann F, Kool M, Wechsler-Reya RJ, Chavez L, Krijgsveld J, Jager N, Pfister SM, Witt O and Milde T. Reduced chromatin binding of MYC is a key effect of HDAC inhibition in MYC amplified medulloblastoma. Neuro Oncol 2021; 23: 226-239.

[42] Nebbioso A, Carafa V, Conte M, Tambaro FP, Abbondanza C, Martens J, Nees M, Benedetti R, Pallavicini I, Minucci S, Garcia-Manero G, Iovino F, Lania G, Ingenito C, Belsito Petrizzi V, Stunnenberg HG and Altucci L. c-Myc modulation and acetylation is a key HDAC inhibitor target in cancer. Clin Cancer Res 2017; 23: 2542-2555.

[43] Faiola F, Liu X, Lo S, Pan S, Zhang K, Lymar E, Farina A and Martinez E. Dual regulation of c-Myc by p300 via acetylation-dependent control of Myc protein turnover and coactivation of Myc-induced transcription. Mol Cell Biol 2005; 25: 10220-10234.

[44] Pei Y, Liu KW, Wang J, Garancher A, Tao R, Esparza LA, Maier DL, Udaka YT, Murad N, Morrissy S, Seker-Cin H, Brabetz S, Qi L, Kogiso M, Schubert S, Olson JM, Cho YJ, Li XN, Crawford JR, Levy ML, Kool M, Pfister SM, Taylor MD and Wechsler-Reya RJ. HDAC and PI3K antagonists cooperate to inhibit growth of MYC-driven medulloblastoma. Cancer Cell 2016; 29: 311-323.

[45] Kim SJ, Kim UJ, Yoo HY, Choi YJ and Kang KW. Anti-cancer effects of CKD-581, a potent histone deacetylase inhibitor against diffuse large B-cell lymphoma. Int J Mol Sci 2020; 21: 4377.
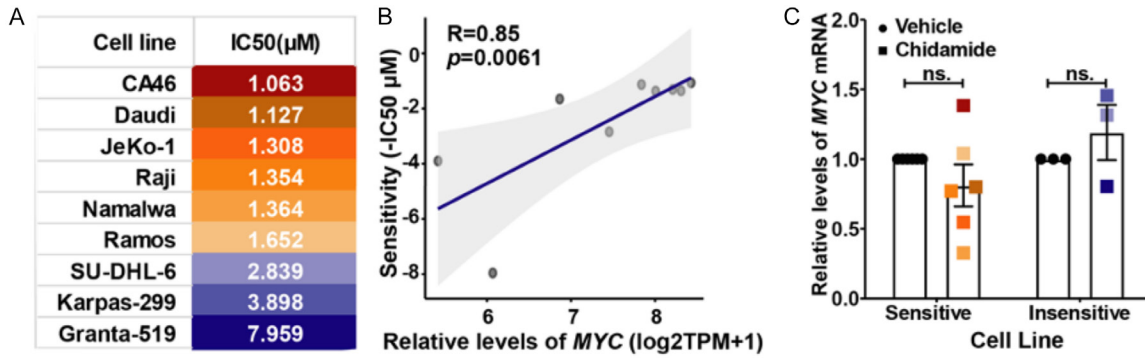
Supplementary Figure 1. The sensitivity of B cell lymphoma cells to chidamide significantly positively correlated with MYC expression level. A. Lymphoma cell lines are grouped into chidamide-sensitive (Red) and chidamide-insensitive (Blue) cell lines according to IC50. B. The correlation between chidamide IC50 and MYC expression levels analyzed by the CCLE database in different lymphoma cell lines (R=0.85, P=0.0061). C. Quantitation of relative RNA expression for MYC in lymphoma cell lines treated with 2 μM chidamide measured by RT-qPCR.
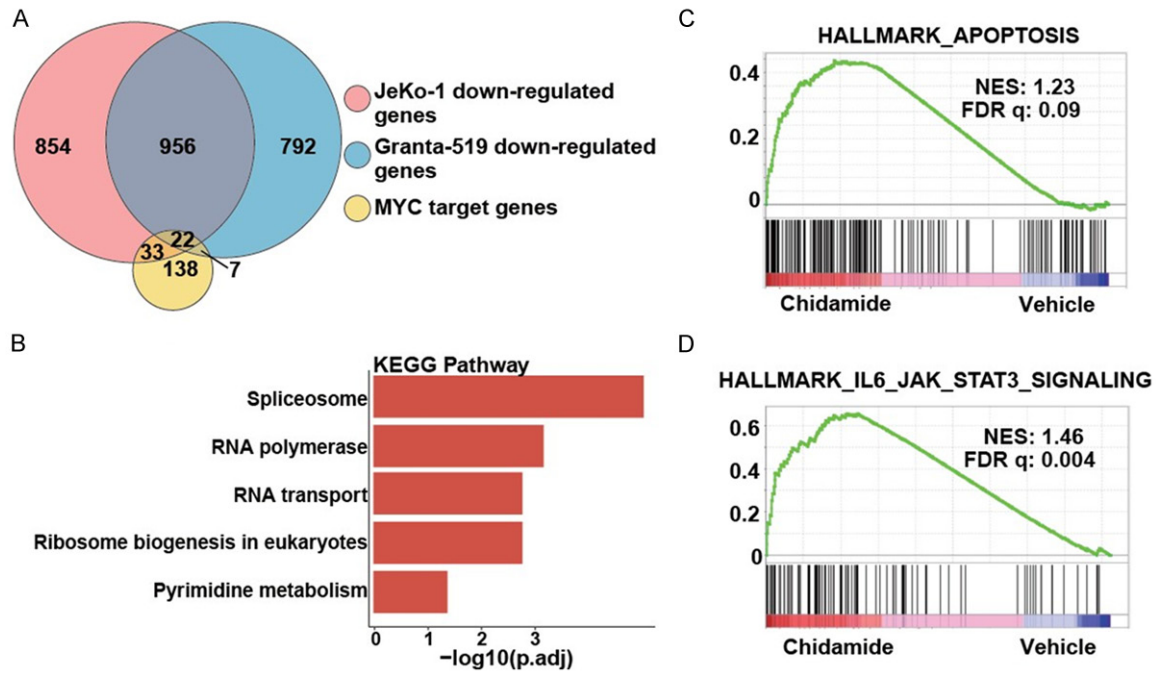
Supplementary Figure 2. Chidamide repress the MYC pathway in B cell lymphoma cell lines. (A) The Venn diagram shows the overlap of down-regulated genes in the chidamide-treated JeKo-1 and Granta-519 with the MYC target genes (common downregulated genes(22) in JeKo-1 and Granta-519 overlap with MYC target genes, P=3.3e-4; JeKo-1 unique down-regulated genes(33) with MYC target genes, P=4.2e-14; Granta-519 unique down-regulated genes(7) overlap with MYC target genes, P=0.69). (B) Bar plots shows the most enriched KEGG pathways in the JeKo-1 unique down-regulated genes treated with chidamide. (C, D) Gene set enrichment analysis (GSEA) shows that the HALLMARK APOPTOSIS pathway (C) and HALLMARK IL6 JAK STAT3 SIGNALING pathway (D) were enriched in the chidamide-treated Jeko-1 cells.
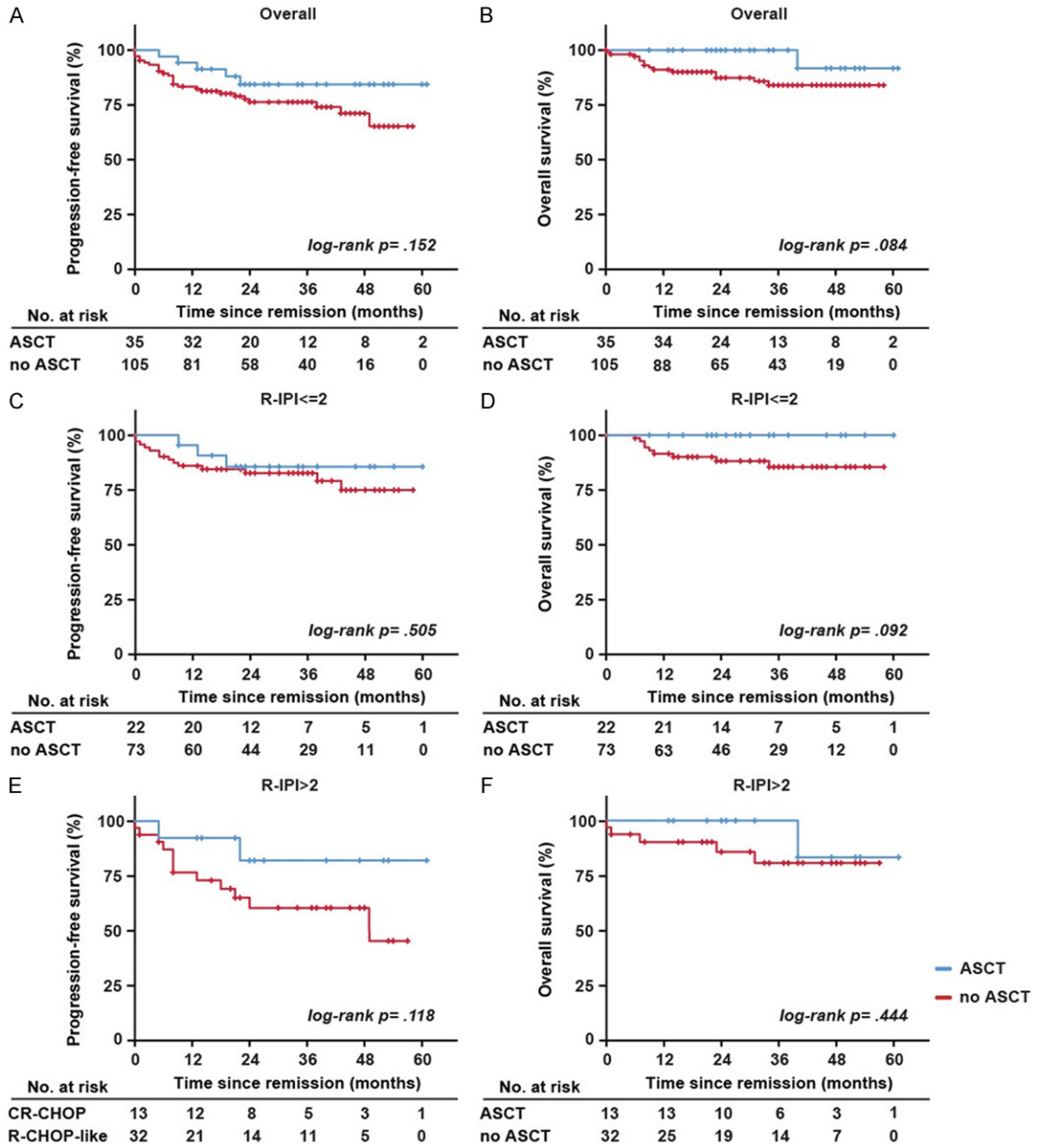
Supplementary Figure 3. Effect of ASCT on the survival of DEL patients after remission. ASCT had a trend to have better progression-free survival (A, C, E) and overall survival (B, D, F) for all DEL patients (A, B), patients with R-IPI ≤2 (C, D) and patient with R-IPI >2 (E, F) after remission. ASCT: Autologous stem cell transplantation.