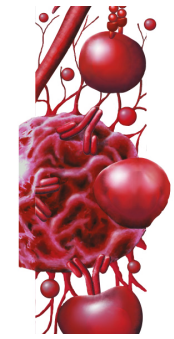
Case Report

Immunotherapy



Progressive diffuse large B-cell lymphoma with *TP53* gene mutation treated with chidamide-based chemotherapy

Qing Li[1], Jingcao Huang[1], Yang Ou[1], Yan Li[1] & Yu Wu*,[1]

[1]Department of Hematology & Hematology Research Laboratory, West China Hospital, Sichuan University, Chengdu, Sichuan 610041, PR China

*Author for correspondence: Tel.: +86 28 8542 2370; Fax: +86 28 8542 3921; wu_yu@scu.edu

We attempted to explore novel treatment options for progressive diffuse large B-cell lymphoma (DLBCL) with *TP53* mutation that has a poor response to rituximab-based immunochemotherapy. Herein, we report the case of a patient with DLBCL having *TP53* mutation who showed progression following four cycles of rituximab-based immunochemotherapy but achieved sustained partial remission following chidamide-based chemotherapy. *In vitro* experiments performed using the DLBCL cell lines OCI-ly1 (LY1; mutant *TP53*), OCI-ly10 (LY10; wild-type *TP53*) and OCI-ly19 (LY19, wild-type *TP53*) demonstrated that chidamide is more potent against cells with mutant *TP53* mutant than those with wild-type *TP53*. Moreover, chidamide can reduce the mRNA and protein expression levels of mutant *TP53* and upregulate the surface expression of the CD20 antigen in lymphoma cells.

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Keywords: CD20 • chidamide • diffuse large B-cell lymphoma • immunochemotherapy • immunomodulator • thalidomide • *TP53* mutation

Presentation & initial diagnosis

The patient was a 74-year-old man who was diagnosed with diffuse large B-cell lymphoma (DLBCL) nongerminal center subtype (immunohistochemistry) stage III using lymph node biopsy. Immunohistochemical analysis of tumor cells indicated positivity for CD20, BCL-6 and MUM-1. The positivity of Ki-67/MIB, C-MYC and BCL-2 were 70, 20 and 80%, respectively. Tumor cells were negative for CD3, CD10, CD30, GB, TIA-1/ALK-1 and EMA (Figure 1). The fluorescence *in situ* hybridization performed suggested that the *BCL -2* gene copy number was increased, but there were no *BCL-2, BCL-6* or *C-MYC* gene translocations. Additionally, PET-CT conducted revealed multiple lymphadenopathies in the neck, abdomen and spleen areas (Figure 2).

Treatment

The patient was treated using R-CHOP (rituximab 375 mg/m^2 intravenous infusion [iv.], D1; cyclophosphamide 750 mg/m^2 [iv.], D2; vincristine 1.4 mg/m^2 [iv.], D2; doxorubicin hydrochloride 50 mg/m^2 [iv.], D2; and prednisone 50 mg p.o. b.i.d., D2, 3, 4, 5 and 6). He experienced several pulmonary infections during postchemotherapy myelosuppression. Following four ccles of immunochemotherapy, the lymph nodes re-enlarged, and he experienced abdominal pain. PET-CT revealed newly emerging lymph nodes in the neck and abdomen (Deauville score, 5 points) and enlargement of the para-aortic lymph nodes with increased glucose metabolism (Figure 2). Considering the progressing disease and the vulnerable status of the patient, ctDNA technology (sequencing platform, Illumina; detection method, NGS; Wuhan Baitai Medical Laboratory, Wuhan, China; coverage depth, up to 4218×) was used as previously described by Alizadeh [1] and Rossi [2], it detected a *TP53* gene mutation at site P.R248Q, with a variant allele fraction of 1.3%. Chidamide-based chemotherapy was administered. The specific chemotherapy regimen included rituximab 375 mg/m^2 iv. monthly, chidamide 30 mg p.o. b.i.w., prednisone 20 mg p.o. q.o.d., cyclophosphamide 50 mg p.o. q.o.d. and thalidomide 50 mg p.o. q.n. After 2 months of chidamide-based chemotherapy, the patient's lymph nodes unexpectedly shrank to an inaccessible size, and the regimen was continued. PET-CT was performed 3 months later to evaluate the response to the novel regimen. Except for stable

Future Medicine

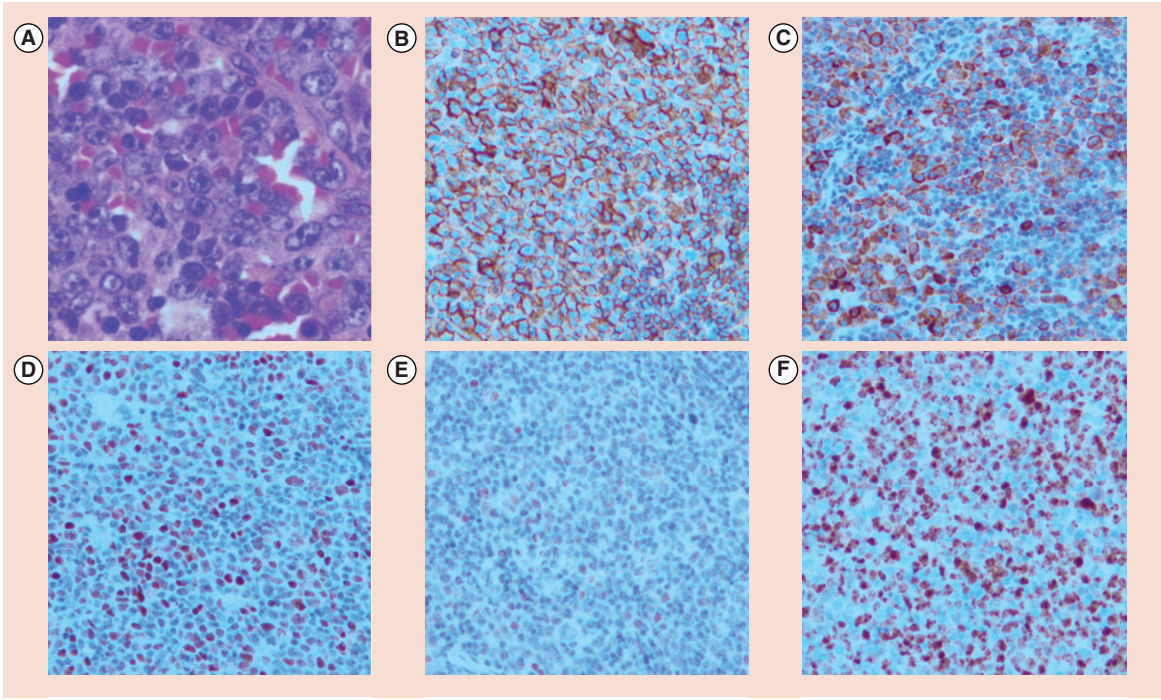

Figure 1. Lymph node biopsy and immunohistochemical staining. (A) Lymph node biopsy (hematoxylin and eosin, ×400); **(B)** positive for CD20; **(C)** positive for BCL-2 (+, 80%); **(D)** positive for BCL-6; **(E)** positive for C-MYC (+, 20%); **(F)** positive for P53 (+, >90%) (B–F, ×200).

findings in lymph nodes in the right cervical region, the glucose metabolism levels in the remaining lymph nodes showed reductions, and no lesions were observed in the left cervical or para-aortic lymph nodes (Figure 2).

Outcome

The patient has survived for >1 year since the date of diagnosis. He is in a good condition with no new lesions, and partial remission has been maintained for up to 9 months. Adverse reactions included mild fatigue and gastrointestinal discomfort among others.

In vitro experiments

We performed *in vitro* experiments to determine whether chidamide is more potent against cells with mutant *TP53* than those with wild-type *TP53*.

Methods

Cell lines

The transformed DLBCL cell lines OCI-ly1 (LY1) (mut*TP53*), OCI-ly10 (LY10) (wt*TP53*) [3] and OCI-ly19 (LY19) (wt*TP53*) [4] were obtained from the State Key Laboratory of Biotherapy, Sichuan University, Sichuan, China.

Written informed consent was obtained from the patient, and the study protocol was approved by the Ethics Committee. The study was performed in accordance with the Declaration of Helsinki.

Cell viability assay

Cell viability was determined using an MTT proliferation assay kit (Biosharp Life Sciences, Anhui, China), according to the manufacturer's instructions. Chidamide was tested at concentrations from 0.1 to 25 μmol/l to determine growth inhibition at 48 or 72 h after treatment in all cell lines. The concentration of the drug that inhibited 50% of cells (IC_{50}) was calculated. The experiments were repeated twice, and each sample had three duplications.

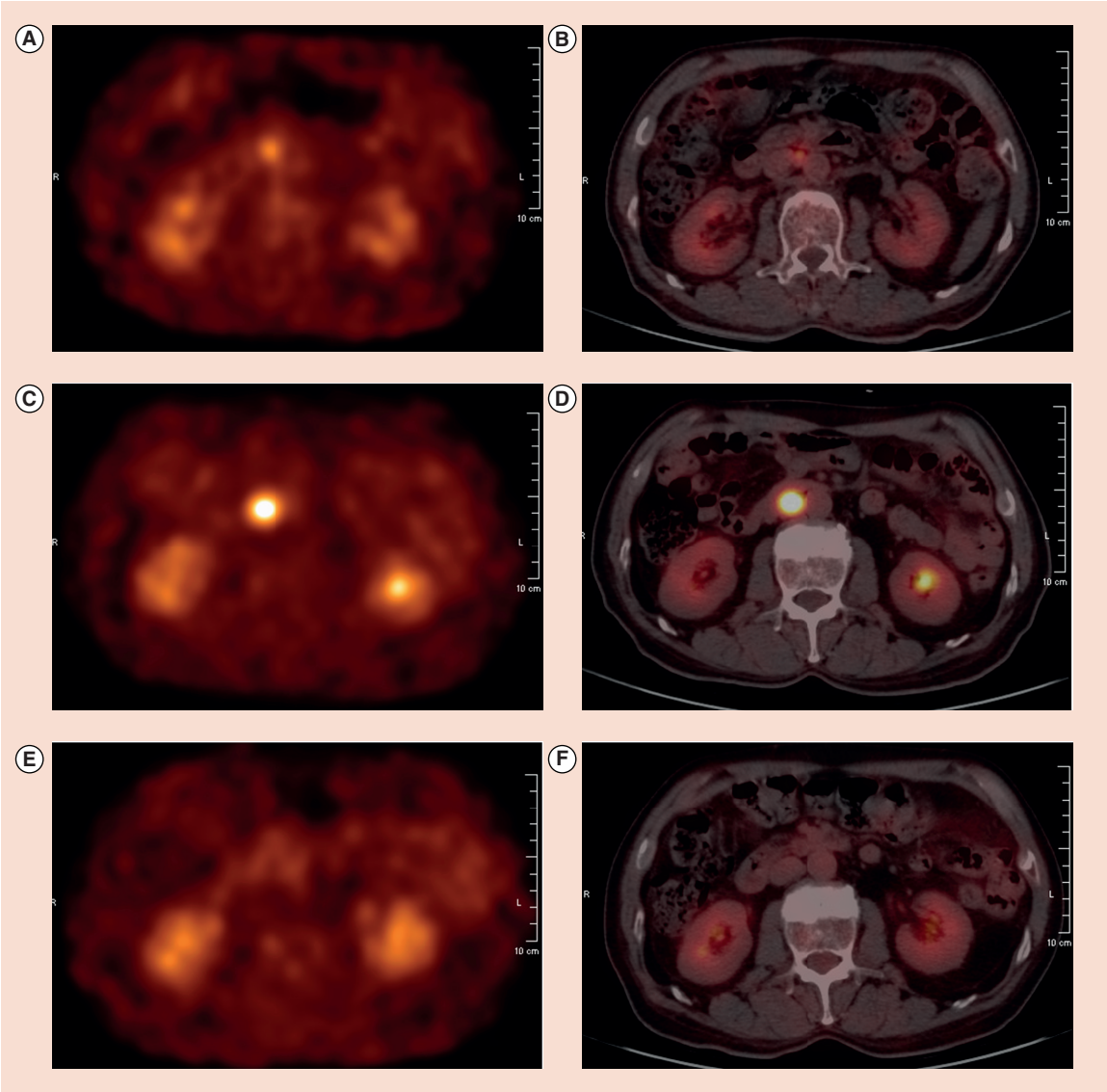

Figure 2. PET-CT results of the patient at the initial diagnosis, during disease progression and at the final follow-up evaluation following chidamide-based chemotherapy. (A & B) PET-CT image of the patient at the initial diagnosis shows lymphoma invasion. **(C & D)** Following four cycles of rituximab-based immunochemotherapy, additional PET-CT shows that the para-aortic lymph nodes are larger than before, with a higher level of glucose metabolism, indicating disease progression. **(E & F)** After 4 months of a chidamide-based chemotherapy regimen, another PET-CT shows no lesions in the para-aortic lymph nodes. Overall, the patient shows sustained partial remission.

Real-time polymerase chain reaction assay

Total RNA was extracted from cell lines using Trizol reagent (MRC, OH, USA), according to the manufacturer's instruction. The expression of target genes was analyzed with real-time quantitative polymerase chain reaction using the SYBR green real-time PCR system (Promega, Shanghai, China). The expression of the housekeeping gene *GAPDH* was used as an internal control. The primers used for *TP53* were 5′-ACCTATGGAAACTACTTCCTGAAAA-3′ and 5′-CAATATCGTCCGGGGACAGC-3′ separately. The primers used for *GAPDH* were 5′-CGCTGAGTACGTCGTGGAGTC-3′ and 5′-GCTGATGATCTTGAGGCTGTTGTC-3′ separately.

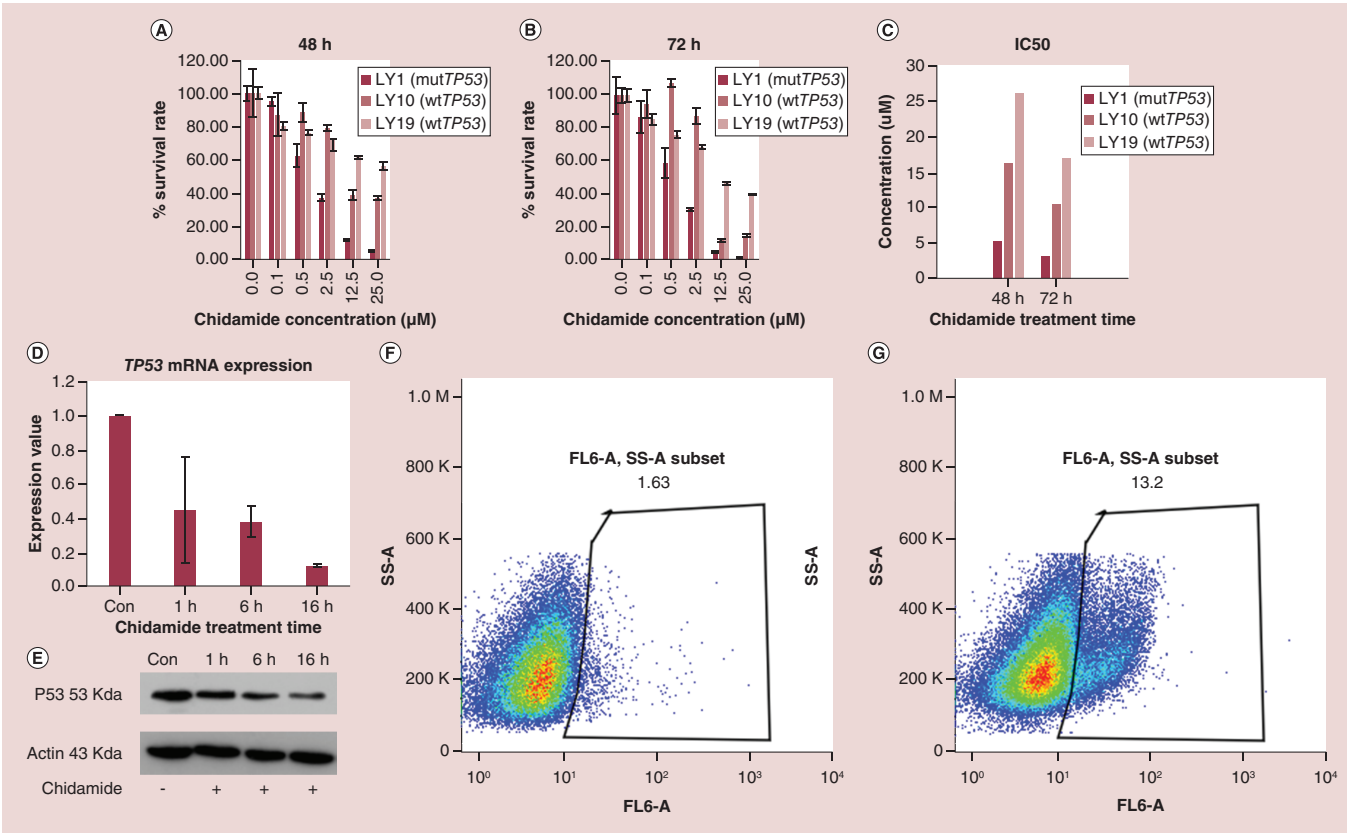

Figure 3. ***In vitro*** **experiments involving chidamide treatment in OCI-ly1 (LY1) (mut*TP53*), OCI-ly10 (LY10) (wt*TP53*) and OCI-ly19 (LY19) (wt*TP53*) lymphoma cell lines. (A & B)** On chidamide treatment in the three lymphoma cell lines for 48 and 72 h, the survival rate of LY1 (mut*TP53*) cells is lower than the rates of LY10 (wt*TP53*) and LY19 (wt*TP53*) cells ($p < 0.05$). **(C)** Following chidamide treatment in the three lymphoma cell lines for 48 and 72 h, the half-maximal inhibitory concentration (IC_{50}), calculated using SPSS software, of LY1 (mut*TP53*) cells is lower than the values of LY10 (wt*TP53*) and LY19 (wt*TP53*) cells ($p < 0.05$). **(D)** LY1 (mut*TP53*) cells are treated with 5.0 μM chidamide (IC_{50}, 48 h) for 1, 6 and 16 h, and the cells are then harvested for real-time quantitative polymerase chain reaction analysis. The expression of *TP53* mRNA shows a decrease with the extension of the chidamide treatment time. **(E)** LY1 (mut*TP53*) cells are treated with chidamide for 1, 6 and 16 h before collection and protein extraction. Western blot analysis performed reveals that chidamide reduces the protein expression of mutant *TP53*. **(F & G)** The expression of the CD20 in LY1 (mut*TP53*) cells treated with chidamide (5.0 μM) for 72 h is compared with that in control cells using flow cytometry. **(F)** The expression rate of the CD20 in LY1 (mut*TP53*) cells in the control group is 1.63%. **(G)** The expression rate of the CD20 in LY1 (mut*TP53*) cells in the treatment group is 13.2%.

Western blot analysis

Western blot analysis was performed according to a standard method [5] using the anti-P53 antibody (Santa Cruz Biotechnology, CA, USA) and anti-β-actin antibody (4A Biotech, Beijing, China). Finally, reactive proteins were visualized using a chemiluminescence kit (Millipore, MA, USA).

Flow cytometry analysis

Following drug treatment, the cells were processed according to a standard method, which involved incubation with anti-CD20 antibody (BD, Pharmingen, Shanghai, China), and the results were analyzed using a Navious flow cytometer (Beckman Coulter, CA, USA).

Results

Chidamide is more potent against DLBCL cells with mutant TP53 than those with wild-type *TP53*

The survival rate of LY1 (mut*TP53*) cells was lower than the rates of LY10 (wt*TP53*) and LY19 (wt*TP53*) cells following drug treatment for 48 or 72 h (Figure 3A & B); the differences were statistically significant ($p < 0.0001$). On treating the cell lines with chidamide concentrations of 12.5 and 25.0 μM for 48 h, the survival rates were 11.84 and 5.51% for LY1 (mut*TP53*) cells, 39.14 and 37.04% for LY10 (wt*TP53*) cells and 61.41 and 56.16% for LY19 (wt*TP53*) cells, respectively. The IC_{50} was calculated using SPSS Statistics software (IBM, NY, USA).

After 72 h of treatment, the IC_{50} of LY1 (mut*TP53*) cells (3.007 μM) was lower than the values of LY10 (wt*TP53*) (10.452 μM) and LY19 (wt*TP53*) cells (16.916 μM) (Figure 3C).

Chidamide inhibited the transcription & translation of mutated *TP53*

LY1 (mut*TP53*) cells were treated with chidamide 5.0 μM (IC_{50} value at 48 h) for 1, 6 and 16 h. The cells were collected at various time points to extract RNA and protein. Real-time quantitative polymerase chain reaction and western blot analysis showed that the inhibition of *TP53* was time-dependent both at the mRNA and protein levels (Figure 3D & E).

Chidamide upregulated the surface expression of the CD20 antigen in lymphoma cells

Following treatment with chidamide (5 μM) for 72 h, the expression rate of the CD20 on LY1 (mut*TP53*) cells was 13.2%, whereas the expression rate on untreated cells was only 1.63% (Figure 3F & G).

Discussion

DLBCL is the most common non-Hodgkin's lymphoma worldwide and is biologically aggressive [6]. *TP53* mutations are the most common mutations in DLBCL and account for 20–25% of cases [7,8]. The prognostic significance of *TP53* mutations in patients with DLBCL has been reported in many studies [9]. A *TP53* mutation is often investigated in patients with R-CHOP failure and is considered to be an adverse factor in DLBCL [10]. A previous study has reported that the rates of complete remission, overall survival and survival at 5 years were significantly lower in patients with tumors having *TP53* mutations than in those with tumors having the wild-type *TP53* [11]. Here, we reported a case of patient with DLBCL having *TP53* mutation that was resistant to R-CHOP. Partial durable relief was obtained using a 2-month chidamide-based regimen. Moreover, the *in vitro* experiments demonstrated that chidamide has a greater lethal effect on DLBCL cell lines with mutant *TP53* than on cell lines with wild-type *TP53*. Furthermore, chidamide has a therapeutic effect on DLBCL and may act by inhibiting the transcription and translation of mutated *TP53* and upregulating the surface expression of the CD20 antigen in lymphoma cells. To the best of our knowledge, this is the first reported case of refractory DLBCL showing a durable response to chidamide treatment.

Currently, the standard chemotherapy regimen for CD20-positive DLBCL is R-CHOP. Despite the invasive nature of this disease, 50–70% of patients can be cured using this regimen [12]. However, the R-CHOP regimen is not suitable for the remaining 30–40% of patients owing to tumor biological heterogeneity, host microenvironment complexity, drug resistance and other factors. Although certain mechanisms are involved in R-CHOP resistance, most patients with DLBCL present double-hit lymphoma, which involves double rearrangement of the *C-MYC* and *BCL-2,* or double-expression lymphoma, which shows high protein expression of both *C- MYC* and *BCL-2* [13]. However, the present patient did not have double-hit lymphoma or double-expression lymphoma. This prompted us to consider other possible mechanisms for R-CHOP resistance. Scherer *et al*. have demonstrated that the mutation analysis of ctDNA can reveal the biological factors involved in the clinical outcome of lymphoma and help in the selection of individualized treatment [14]. Molecular monitoring of ctDNA has great potential and may be useful for monitoring the response to therapy or early relapse in various types of invasive B-cell lymphomas [15]. Through the regulation of downstream target genes, wild-type *TP53* governs major defenses against tumor growth and promotes apoptosis, cellular DNA repair and cell cycle arrest [16]. On the other hand, mutant *TP53* causes the loss of proliferation control, cell cycle dysregulation and genomic instability [17,18]. Following the progression of lymphoma in the current patient, a ctDNA test was performed, and it indicated the presence of a *TP53* gene mutation that was clinically significant. This finding played a critical role in the subsequent decision-making process.

There is no consensus on the treatment of relapsed/refractory DLBCL with *TP53* mutation. HDAC1 and HDAC2 can maintain the expression of mutant *TP53*, and small-molecule HDAC inhibitors can reduce the transcription and protein expression of mutant *TP53* [19]. A Phase II study performed to evaluate the efficacy and safety of chidamide in Chinese patients with relapsed or refractory peripheral T-cell lymphoma has shown that chidamide acts as a novel benzamide-type subtype-selective HDAC inhibitor, with remarkable single-drug activity and controllable toxicity, and it provides a new therapeutic option for patients with peripheral T-cell lymphoma [20]. Moreover, clinical trials of chidamide for DLBCL are currently underway, and we look forward to encouraging results. HDAC inhibitors can overcome congenital and acquired rituximab resistance in patients with various types of B-cell lymphoma and augment the cytotoxic activity of rituximab by upregulating the expression of the CD20

in lymphoma cells [5]. Chidamide, the new HDAC inhibitor available in China, would be an option for refractory DLBCL. Coleman *et al.* have used the PEP-C regimen (prednisone 20 mg, etoposide 50 mg/m^2, procarbazine 50 mg/m^2 and cyclophosphamide 50 mg/m^2) to treat 75 cases of relapsed and refractory lymphomas in a clinical trial. The response rate for refractory DLBCL is 3/9 and the time on treatment ranged from 3 weeks to 48 months (median 10 months). They have shown that the PEP-C regimen represents an active, tolerable treatment approach in patients with recurrent lymphoma [21]. This metronomic regimen might have been suitable for the present patient who was frail and refractory to current therapy and who needed control of accelerating lymphoma growth in a placid slope. Additionally, chidamide can induce mutant *TP53* mRNA and protein drop in a time-dependent manner.

Thalidomide and lenalidomide are potent antineoplastic agents for myeloma as well as lymphoma. A Phase II clinical trial comparing thalidomide plus CHOP with CHOP alone has shown that the combination involving thalidomide significantly increased the complete response (CR) rate, particularly in patients with BCL-2-positive and BCL-6-negative DLBCL [22]. A case study has shown that two out of three relapsed elderly patients with DLBCL achieved complete remission using the combination of thalidomide 100 mg and corticosteroids [23]. Two Phase II trials have shown that the addition of lenalidomide to R-CHOP appeared to mitigate the negative impact of the non-germinal center B-cell (GCB) phenotype on DLBCL outcome [24,25]. There are limited data on the effect of lenalidomide in relapsed or refractory DLBCL. Wang has reported the response rate of R2 regimen (rituximab plus lenalidomide) in relapsed DLBCL was about 28% with median 10.2 months OS [26]. The present patient experienced an allergic reaction on administration of lenalidomide; therefore, treatment was shifted to thalidomide.

Conclusion

Based on the efficacy and tolerability of the metronomic regimen, the possible mechanisms to overcome *TP53* mutation through HDAC inhibitors, and *in vitro* experiment results, we believe that chidamide is a novel agent for treating patients with refractory DLBCL having *TP53* mutation. We reported a progressive DLBCL with *TP53* mutation that obtained sustained partial remission based on chidamide in combination with metronomic regimen. However, further clinical trials and experimental investigations are needed.

Summary points

- Diffuse large B-cell lymphoma (DLBCL) with *TP53* mutation is often resistant to rituximab-based immunochemotherapy (R-CHOP) and has a poor prognosis.
- We report a case of a patient with R-CHOP-resistant senile DLBCL having *TP53* mutation who showed sustained remission following treatment using a chidamide-based chemotherapy regimen.
- Furthermore, *in vitro* experiments demonstrated that chidamide has a greater lethal effect on DLBCL cell lines with mutant *TP53* than on those with wild-type *TP53*. Moreover, chidamide has a therapeutic effect on DLBCL and may act by inhibiting the transcription and translation of mutated *TP53* and upregulating the surface expression of the CD20 antigen in lymphoma cells.
- The chidamide-based chemotherapy regimen may be a new treatment option in patients with progressive DLBCL having *TP53* mutation.

Financial & competing interests disclosure

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No writing assistance was utilized in the production of this manuscript.

Ethical disclosure

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or *in vitro* experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved. The authors state that they have obtained verbal and written informed consent from the patient/patients for the inclusion of their medical and treatment history within this case report.

Author contributions

Q Li wrote the manuscript, Q Li and J Huang processed the *in vitro* experiment, Y Ou and Y Li collected the clinical data and did the follow-up. Y Wu designed the study and directed the project.

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