The outcome of ibrutinib-based regimens in relapsed/refractory central nervous system lymphoma and the potential impact of genomic variants

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Advances in Clinical and Experimental Medicine, ISSN 1899–5276 (print), ISSN 2451–2680 (online) *Adv Clin Exp Med*. 2023;32(8):855–863

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Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
This work was supported by the National Natural Science Foundation of China (grant No. 81870081), Program for Outstanding Medical Academic Leader of Shanghai (grant No. 2019LJ05), Shanghai Collaborative Innovation Program on Regenerative Medicine and Stem Cell Research (grant No. 2019CXJQ01), and Translational Research Grant of the National Clinical Research Center for Hematologic Diseases (No. 2020ZKMB03), all awarded to Dr. Tong Chen. Additionally, we thank Professor Xiaolei Lin (Fudan University) for her kind help on statistical analysis.

Received on October 15, 2022
Reviewed on January 9, 2023
Accepted on January 13, 2023

Published online on March 7, 2023

Cite as
Wang S, Zhu YQ, Qian XH, et al. The outcome of ibrutinib-based regimens in relapsed/refractory central nervous system lymphoma and the potential impact of genomic variants. *Adv Clin Exp Med*. 2023;32(8):855–863. doi:10.17219/acem/159288

DOI
10.17219/acem/159288

Abstract

Background. Relapsed/refractory (r/r) central nervous system lymphoma (CNSL) exhibits aggressive behavior and poor outcomes. As an effective bruton tyrosine kinase (BTK) inhibitor, ibrutinib yields benefits in B-cell malignancies.

Objectives. We aimed to explore the efficacy of ibrutinib in treating r/r CNSL patients, and whether genomic variants impact treatment outcomes.

Materials and methods. The ibrutinib-based regimens in 12 r/r primary CNSL (PCNSL) and 2 secondary CNSL (SCNSL) patients were analyzed retrospectively. The impact of genetic variants on the effects of treatments was examined using whole-exome sequencing (WES) technology.

Results. In PCNSL, the overall response rate was 75%, with median overall survival (OS) not reached (NR) and progression-free survival (PFS) of 4 months. Both SCNSL patients responded to ibrutinib, with median OS NR and PFS of 0.5–1.5 months. Infections were common during ibrutinib therapy (42.86%). The PCNSL patients harboring gene mutations in *PIM1*, *MYD88* and *CD79B*, and the proximal BCR and nuclear factor kappa B (NF-κB) pathways responded to ibrutinib. Patients who harbored simple genetic variants and those with a low tumor mutation burden (TMB; 2.39–5.56/Mb) responded swiftly and maintained remission for more than 10 months. A patient with a TMB of 11/Mb responded to ibrutinib but continued to experience disease progression. In contrast, patients with complex genomic features, especially extremely high TMB (58.39/Mb), responded poorly to ibrutinib.

Conclusions. Our study demonstrates that ibrutinib-based therapy is effective and relatively safe for the treatment of r/r CNSL. Patients with less genomic complexity, especially with regard to TMB, might benefit more from ibrutinib regimens.

Key words: ibrutinib, tumor mutation burden, central nervous system lymphoma, relapsed/refractory, genomic variants

Background

Central nervous system lymphoma (CNSL) is a type of extra-nodal lymphoma that most commonly manifests as primary CNSL (PCNSL) and secondary CNSL (SCNSL). Primary CNSL is a rare and aggressive type of lymphoma, 95% of which are diffuse large B cell lymphoma (DLBCL),[1] while SCNSL refers to systematic DLBCL that has spread to the CNS. The backbone of PCNSL treatment is high-dose methotrexate (HD-MTX) in combination with other agents.[2] However, due to the blood–brain barrier and the special immune microenvironment of the CNS, CNSL tends to show more aggressive behavior than systematic DLBCL, resulting in a poorer prognosis for relapsed/refractory (r/r) patients under standard treatments.[3]

Bruton tyrosine kinase (BTK), which mediates signals from the B-cell antigen receptor (BCR), toll-like receptor (TLR) and downstream pathways, plays an irreplaceable role in the survival of B cells.[4] In recent years, the first-generation BTK inhibitor ibrutinib has been used to treat CNSL,[5] yielding promising results with an acceptable safety profile across its combination regimens and single-agent-targeted therapy for r/r CNSL. For patients who fail to respond to traditional treatments,[6–9] this offers a great alternative treatment option.

Although mutations of *MYD88* and *CD79B* are widely accepted as hallmarks of DLBCL in immune-privileged sites due to their key roles in the BCR and TLR signaling pathways, there has been no consensus regarding their effects on ibrutinib treatment.[10,11] Some studies have claimed that *CD79B* mutations weaken the response to BTK inhibitors,[9] while others have reported that *MYD88*, *CD79B* or BCR pathway mutations have no effects on the tumor response to ibrutinib.[7,8]

Based on the distinct molecular subtypes of DLBCL,[12] a massive prospective study has recently demonstrated that 100% of MCD or N1 subtype DLBCL cases responded to R-CHOP regimes combined with ibrutinib rather than placebo.[13] This highlights the significant likelihood of a potential relationship between genome variants and BTK inhibitor response.

Objectives

In this single-center retrospective study of a CNSL cohort, we aimed to evaluate the effectiveness and side effects of ibrutinib-based regimens, and uncover relationships between clinical outcomes and genomic variants.

Materials and methods

Patients

The r/r CNSL patients who started ibrutinib-based regimens at Huashan Hospital (Shanghai, China) from 2018 to 2020 were enrolled in this single-center retrospective study. The study included 12 r/r PCNSL and 2 r/r SCNSL patients. All patients received enhanced brain magnetic resonance imaging (MRI), whole-body positron emission tomography (PET) or computed tomography (CT) scans, cerebral spinal fluid evaluation, and bone marrow aspiration to determine whether they had primary or secondary CNSL. The responses to treatments were evaluated according to the International PCNSL Collaborative Group (IPCG) guidelines.[14] The National Cancer Institute Common Terminology Criteria for Adverse Events (v. 5.0) were used to assess adverse events (AEs) during ibrutinib therapy. This retrospective cohort study was approved by the Institutional Review Board of Huashan Hospital (Shanghai, China; approval No. KY2020-879). The study complies with the Declaration of Helsinki (as revised in 2013). Informed consent was obtained individually from the included patients.

Whole-exome sequencing and data processing

Exon regions were captured using SureSelect Exome arrays (Agilent Technologies, Santa Clara, USA) and sequenced with Hiseq System (Illumina, San Diego, USA). Paired-end fastq reads were mapped to University of California Santa Cruz (UCSC) human genome hg19 using BWA software (https://hpc.nih.gov/apps/bwa.html).[15] Mutations were called by the Genome Analysis Toolkit (GATK v. 4.1.2) and Mutect (v. 2) workflow (Broad Institute, Cambridge, USA).[16] All mutations were annotated by ANNOVAR software (http://annovar.openbioinformatics.org).[17] Facets software was used to calculate somatic copy number variation (CNV).[18] The R package "sigminer" (R Foundation for Statistical Computing, Vienna, Austria) was applied to analyze mutation signature.[19]

Pipeline filtering paired tumor samples had the following characteristics: 1) at least ×10 coverage, variant allele frequency (VAF) ≥ 5% and altered reads ≥3; 2) all variants had less than 0.0025 VAF in a matched normal sample; 3) variants located in exon or splice were retained; 4) nonsynonymous somatic nucleotide variant (SNV) was required; 5) variants with a frequency over 1% in 1000G in the East Asian or East Asian of Genome Aggregation Database (gnomAD_EAS) sites[20,21] and not listed in the COSMIC v. 92 database were excluded.[22]

The criteria for formalin-fixed paraffin-embedded (FFPE) samples were: 1) variants with at least ×20 coverage, VAF ≥ 5% and altered reads ≥3; 2) for variants reads ranging ×10–20, VAF must be higher than 0.2; 3) variants located in exon or splice were retained and nonsynonymous SNV was required; 4) germline variants were filtered including a) variants with a frequency over 1% in both 1000G East Asian or gnomAD_EAS sites, and b) sites not included in COSMIC v. 92 but in the single nucleotide polymorphism database (dbSNP)150 or with VAF ≥ 80%; and 5) C > T and G > T base substitution with VAF < 15% were filtered out to avoid artifacts brought by formalin.[23,24]

Statistical analyses

To reflect the general survival and disease improvement, overall survival (OS) and progression-free survival (PFS) were both calculated. Overall survival was measured from the initiation of ibrutinib therapy until discontinuation or death. Progression-free survival was defined as the time elapsed from the initiation of ibrutinib therapy until disease progression, according to MRI assessment. Since the sample size was small in this cohort, the 95% confidence interval (95% CI) of overall response rate (ORR) was estimated using the Clopper–Pearson method, which works when np > 5 or n(1–p) > 5 (where n – number, p – probability).[25,26] The OS and PFS rates with 95% CIs were calculated using the Kaplan–Meier method.[8,9]

Results

Characteristics of r/r patients treated with ibrutinib-based therapy

To investigate the clinical benefits of the BTK inhibitor, 14 r/r CNSL patients treated with ibrutinib-based regimens were enrolled in this single-center study (12 PCNSL and 2 SCNSL patients). The basic characteristics of these patients are listed in Table 1. The median age was 58 years at diagnosis (37–80 years), with 6 patients (42.86%) aged over 60 years. In the PCNSL cohort, 66.67% were refractory patients. Both SCNSL patients experienced recurrent disease. All patients received a median of 3 lines of therapy (range: 2–4) before being prescribed ibrutinib (Fig. 1A). In the PCNSL cohort, HD-MTX was administered to all 12 patients, rituximab to 10 patients and radiotherapy to 5 patients. Both SCNSL patients received prior R-CHOP treatment. Combined with ibrutinib, 11 PCNSL and both SCNSL patients were treated with HD-MTX-based chemotherapy. One PCNSL patient (PCNSL #11) was not eligible for HD-MTX-based chemotherapy due to MTX-related myelosuppression, and was given ibrutinib combined with cytarabine. In the PCNSL cohort, the median time from the last treatment to ibrutinib initiation was 1.5 months (range: 0.5–5 months). For the 2 SCNSL patients, the interval was 2.5 months and 3.5 months, respectively.

Table 1. Baseline characteristics of relapsed/refractory (r/r) patients before ibrutinib treatment

Characteristics		Values
Age [years]	median	58
	range	37–80
	≥60	6 (42.86%)
Gender, n (%)	male	10 (71.43)
	female	4 (28.57)
ECOG (range)		1 (1–2)
Status from previous treatment, n (%)	primary CNS lymphoma	12 (85.71)
	refractory	8 (66.67)
	recurrent	4 (33.33)
	secondary CNS lymphoma	2 (14.28)
	refractory	0
	recurrent	2 (100)
CNS involvement, n (%)	brain	14 (100)
	eye	1 (7.14)
	cerebrospinal fluid	1 (7.14)
	spinal cord	1 (7.14)
Previous regimens, n (%)	primary CNS lymphoma	12
	HD-MTX	12 (100)
	rituximab	10 (83.33)
	radiation	5 (41.67)
	WBRT	4 (80)
	stem cell transplant	3 (25)
	secondary CNS lymphoma	2
	R-CHOP	2 (100)
	stem cell transplant	1 (50)
	radiation	1 (50)
Median number of prior treatments (range)		3 (2–4)
Ibrutinib-based treatments, n (%)	primary CNS lymphoma	12
	HD-MTX	11 (91.67)
	rituximab	3 (25)
	chemotherapy	4 (33.33)
	secondary CNS lymphoma	2
	HD-MTX	2 (100)
	rituximab	1 (50)
	chemotherapy	1 (50)

ECOG – Eastern Cooperative Oncology Group; HD-MTX – high-dose methotrexate; CNS – central nervous system; WBRT – whole-brain radiotherapy.

Response to ibrutinib-based treatment

The initial dose of ibrutinib was 420 mg/day, and for 4 patients this was later increased to 560 mg/day to address progressive disease. Sulfamethoxazole was administered to prevent infections during ibrutinib treatment. Therapeutic responses were evaluated at a median follow-up time of 6.5 months (range: 2.5–21 months). Among the 12 PCNSL cases, 6 patients achieved complete remission/unconfirmed complete remission (CR/CR(u); 50%) and 3 patients achieved partial remission (PR; 25%) at their best responses (Fig. 1A), yielding an ORR of 75% (9/12; 95% CI: 42.81–94.51%). Both SCNSL patients achieved CR/CR(u) during ibrutinib-based therapy, but the possibility of casuistic information brought by 2 cases only cannot be excluded. Median OS was reached neither in PCNSL nor in SCNSL, as no death occurred during ibrutinib treatment. Median PFS was 4 months (range: 1–17 months, 95% CI, 1.5 months – not reached) in PCNSL. Progression-free survival for the 2 SCNSL patients was 1.5 months and

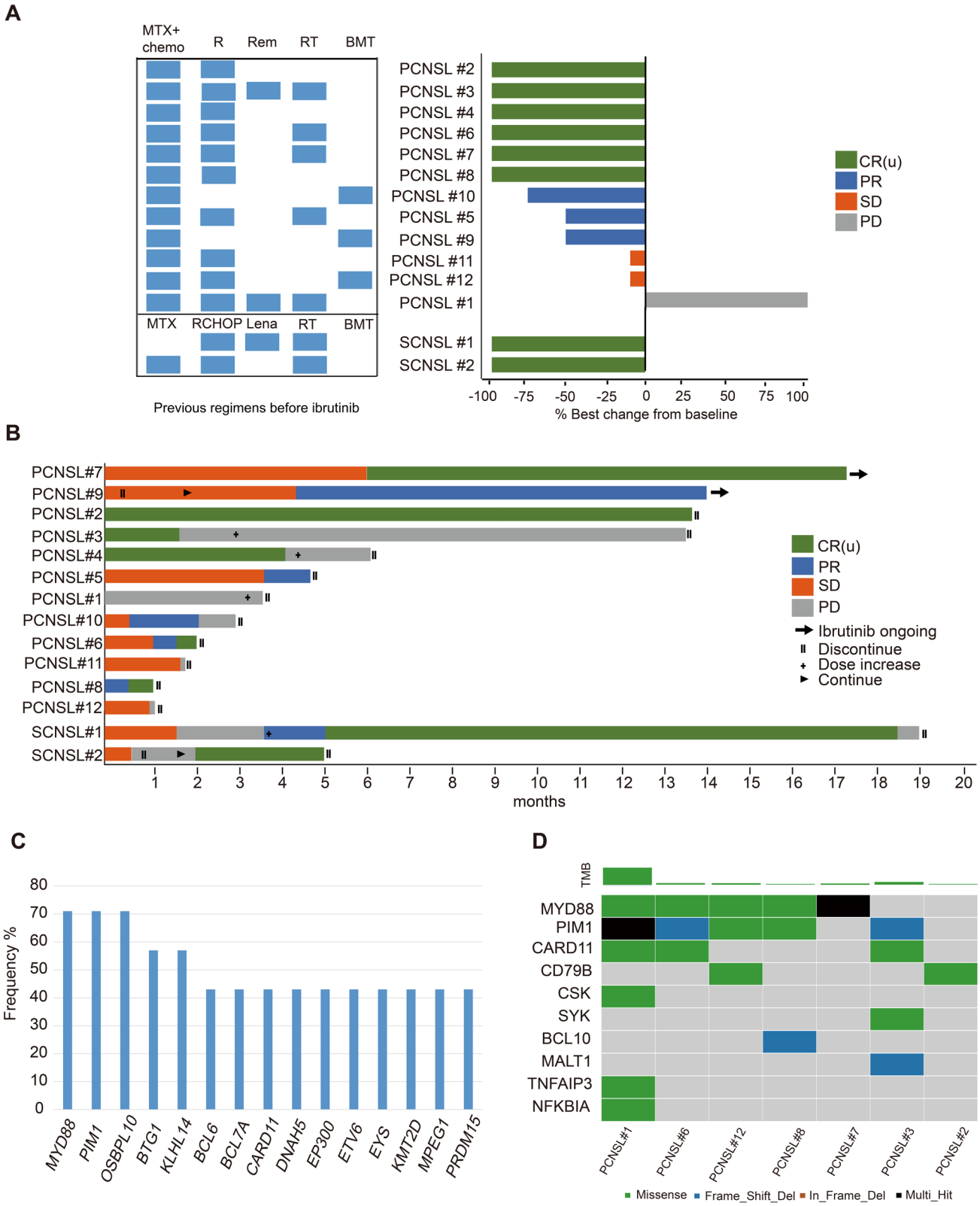

Fig. 1. Response to ibrutinib-based treatment in relapsed/refractory (r/r) primary central nervous system lymphoma (PCNSL) patients. A. Previous regimens before ibrutinib and best responses to ibrutinib-based treatments. Blue diamonds of each column in the light panel indicated therapies experienced (the corresponding column name). Negative bars represent tumor volume shrinkage; B. Response to and duration of ibrutinib-based treatment in a r/r CNSL cohort (n = 14) based on first disease evaluation. The colors of the bars represent the results of tumor assessment. The symbols represent different ibrutinib usage; C. Top gene mutations (over 30%) in PCNSL; D. Mutation profile of the B-cell antigen receptor (BCR) and nuclear factor kappa B (NF-κB) pathways. Each row represents 1 gene and each column represents 1 sample. Types of variants are indicated by different colors of each diamond

SCNSL – secondary central nervous system lymphoma; MTX – methotrexate; chemo – chemotherapy; R – rituximab; Rem – surgical removal; RT – radiation therapy; BMT – bone marrow transplantation; Lena – lenalidomide; CR – complete remission; CR(u) – complete remission unconfirmed; PR – partial remission; SD – stable disease; PD – progressive disease; TMB – tumor mutation burden.

Table 2. Adverse events during ibrutinib treatment (CTCAE v. 5.0)

Adverse events	Number of events (%)			
	grade 1–2	grade 3	grade 4	total
Anemia	7 (50)	1 (7.14)	N/O	8 (57.14)
Platelet count decreased	6 (42.86)	3 (21.43)	N/O	8 (57.14)
Lung infection	N/O	6 (42.86)	N/O	6 (42.86)
White blood cell decreased	3 (21.43)	2 (14.29)	N/O	4 (28.57)
Hypocalcemia	4 (28.57)	N/O	N/O	4 (28.57)
Hyperglycemia	4 (28.57)	N/O	N/O	4 (28.57)
Lymphocyte count decreased	4 (28.57)	N/O	N/O	4 (28.57)
Hypokalemia	3 (21.43)	1 (7.14)	N/O	4 (28.57)
GGT increased	3 (21.43)	N/O	N/O	3 (21.43)
ALP increased	3 (21.43)	N/O	N/O	3 (21.43)
Hematoma	2 (13.33)	N/O	N/O	2 (14.29)
Neutrophil count decreased	2 (14.29)	N/O	N/O	2 (14.29)
Hypoalbuminemia	2 (14.29)	N/O	N/O	2 (14.29)
Headache	1 (7.14)	N/O	N/O	1 (7.14)
Nausea	1 (7.14)	N/O	N/O	1 (7.14)
Diarrhea	1 (7.14)	N/O	N/O	1 (7.14)
Aspartate aminotransferase increased	1 (7.14)	N/O	N/O	1 (7.14)
Abdominal pain	1 (7.14)	N/O	N/O	1 (7.14)
Infectious enterocolitis	N/O	1 (7.14)	N/O	1 (7.14)
Sinus tachycardia	1 (7.14)	N/O	N/O	1 (7.14)
Hearing impaired	1 (7.14)	N/O	N/O	1 (7.14)

CTCAE – Common Terminology Criteria for Adverse Events; GGT – gamma-glutamyl transpeptidase; ALP – alkaline phosphatase; N/O – no occurrence.

0.5 months, respectively. In the PCNSL cohort, the median time to achieve response was 1.5 months (range: 1–6.5 months). For the 2 SCNSLs, it took 4 and 5 months to respond, respectively. During the entire follow-up period, 1/3 of the PCNSL cases and 1 SCNSL case experienced progressive disease (PD) after achieving response. Ibrutinib therapy for the 2 PCNSL cases was continued until the end of follow-up. No influence of previous regimens on the effects of ibrutinib were observed after combining the clinical information before and after the BTK inhibitor for each patient (Fig. 1A). Causes that warranted ibrutinib discontinuation included infections (PCNSL #1, #4, #10), economic reasons (PCNSL #2, #6, #8) and disease progression (PCNSL #3, #10, #11, #12; SCNSL #1, #2). The evaluation of every patient's response during follow-up is presented in Fig. 1B.

Adverse events after ibrutinib-based treatments

As PCNSL is more commonly found in elderly patients, special attention was paid to AEs related to ibrutinib in clinical practice. All AEs that happened in this cohort are listed in Table 2. A total of 14 episodes of grade 3 events were observed. Anemia and low platelet count were the most common AEs (57.14%). Six patients experienced lung infection, 4 of whom were diagnosed with a fungal infection. Three patients discontinued ibrutinib due to persistent infections. One patient withdrew from ibrutinib therapy due to pancytopenia. Other events that occurred in over 20% of patients included a decreased blood cell count (white blood cells and lymphocytes), metabolic disorders (Ca^{2+}, K^{+} and glycemia) and liver insufficiency (increased gamma-glutamyl transpeptidase (GGT) and alkaline phosphatase (ALP)). Neither grade 4 AEs nor atrial fibrillation events were observed during ibrutinib treatment.

Effects of genetic alterations on ibrutinib therapy

Available whole-exome sequencing (WES) data of 7 r/r PCNSL patients at diagnosis were analyzed. Mutations of *MYD88* (71%), *PIM1* (71%), *OSBPL10* (71%), *BTG1* (57%), and *KLHL14* (57%) were most frequently found in this PCNSL cohort (Fig. 1C). Consistent with the key roles of the BCR pathway and the downstream nuclear factor kappa B (NF-κB) pathway in the survival of DLBCL tumor cells,[11] mutations of the proximal BCR pathway and downstream NF-κB pathway were also identified in these 7 PCNSL patients (Fig. 1D). The LymphGen algorithm was then applied to classify the individuals.[12] Molecular subsets of MCD (subtype with *MYD88* L265P and *CD79B* mutations; 2 cases), BN2 (subtype with *BCL6* translocations and

Table 3. Molecular features of relapsed/refractory (r/r) patients treated with ibrutinib-based therapy

Patient	PCNSL #2	PCNSL #7	PCNSL #3	PCNSL #6	PCNSL #8	PCNSL #12	PCNSL #1
Age	69	56	38	66	69	54	51
Ibrutinib duration [months]	15.5	17.5	15	2.5	3	3	5.5
Best response	CR	CR(u)	CR(u)	CR(u)	CR(u)	SD	PD
Response duration [months]	13.5	11	1.5	1	1	–	–
COO	non-GCB	non-GCB	N/O	non-GCB	non-GCB	GCB	non-GCB
TMB [/Mb]	2.39	5.56	11.19	5.90	2.53	5.64	58.39
LymphGen	unclassified	MCD	BN2	MCD/N1	BN2	MCD/EZB	MCD
PIM1	WT	WT	MT	MT	MT	MT	MT
MYD88	WT	L265P P258T	WT	L265P	L142F D148V	L265P	L265P
CD79B	G135A	WT	WT	WT	WT	Y197C	WT
Proximal BCR pathway	WT	WT	SYK	WT	WT	WT	CSK
NF-κB pathway	WT	WT	*CARD11* (C49Y) MALT1	*CARD11* (E588K)	BCL10	WT	*CARD11* (S879L) *TNFAIP3* *NFKBIA*

PCNSL – primary central nervous system lymphoma; CR – complete remission; CR(u) – complete remission unconfirmed; SD – stable disease; PD – progressive disease; COO – cell of origin; GCB – germinal center B-cell like; N/O – no occurrence; TMB – tumor mutation burden; MT – mutation; WT – wild type; BCR – B-cell antigen receptor; NF-κB – nuclear factor kappa B; MCD – subtype with MYD88L265P and CD79B mutations; BN2 – subtype with BCL6 translocations and NOTCH2 mutations; EZB – subtype with EZH2 mutations and BCL2 translocations; N1 – subtype with NOTCH1 mutations.

NOTCH2 mutations; 2 cases), complex phenotype (MCD/N1 (subtype with *NOTCH1* mutations), MCD/EZB (subtype with *EZH2* mutations and *BCL2* translocations)), and 1 unclassified case were obtained using this algorithm (Table 3).

In addition, the relationships between the ibrutinib response and genomic variants in these 7 r/r PCNSL patients were analyzed (Table 3). Patients who harbored mutations in *PIM1*, *MYD88* and *CD79B* responded to ibrutinib-based therapy. Patients with mutations in other genes in the proximal BCR pathway and the NF-κB pathway (such as *CARD11*, *SYK*, *MALT1*, and *BCL10*) also responded to ibrutinib. Based on the LymphGen classification, patients with MCD, BN2, composite (MCD/N1), and unclassified subtypes were all observed to respond to ibrutinib treatment.

Other than gene mutations, the impact of genomic complexity on BTK inhibition was also analyzed, especially in patients with particularly favorable (Fig. 2A,B) and poor (Fig. 2C,D) outcomes. The PCNSL #2 achieved CR(u) soon after 7 weeks of ibrutinib initiation and maintained remission for the entire follow-up (13.5 months; Fig. 2A). Although ibrutinib therapy was discontinued due to economic reasons, the remission of the disease was maintained for another 16 months. The analysis of genomic variants showed a low tumor mutation burden (TMB, 2.39/Mb; Fig. 3). Furthermore, high copy gains of epidermal growth factor receptor (*EGFR*) residing on 7p11.2 were also identified (Fig. 2B). Similarly, PCNSL #7 with a TMB of 5.56/Mb maintained CR(u) for more than 10 months, but required a longer duration to achieve this response (Fig. 3). The PCNSL #6 and #8 harbored a low TMB (5.90/Mb and 2.53/Mb, respectively), and achieved CR(u) to ibrutinib which was maintained for 1 month only. This might have been due to the limited duration of the ibrutinib treatments (2.5 months and 3 months, respectively). For PCNSL #3, who harbored a TMB of 11.19/Mb, the CR response was only maintained for 1.5 months despite ibrutinib treatment being continued for 15 months (Fig. 3). The PCNSL #1, who received surgical resection at diagnosis, relapsed after being administered a rituximab combined with the HD-MTX regimen, and continued to experience PD despite several lines of treatments, including whole-brain radiotherapy (WBRT). Unfortunately, ibrutinib-based therapy (420 mg/day and 560 mg/day) yielded no improvements in his outcome. During the 5.5 months of ibrutinib treatment, a few new lesions emerged, while the original tumors shrank or persisted. Finally, ibrutinib was discontinued due to a fungal lung infection (Fig. 2C). Whole-exome sequencing showed that this patient harbored a high genomic complexity with an extra high TMB of 58.39/Mb (Fig. 3). In addition to multiple alternations in the BCR and NF-κB pathways, this patient also presented a unique genomic signature, exhibiting ultra-hypermutations, similar to the error-prone polymerase *POLE* cancer signatures from the COSMIC database (https://cancer.sanger.ac.uk/cosmic/signatures; Fig. 2D). The mutations of genes involved in DNA repair (*MSH4*, *MSH6* and *POLE*) were also identified in PCNSL #1.

Discussion

This is a retrospective single-center study examining ibrutinib-based regimens in CNSL and exploring the impact of genomic variantson the outcomes of the treatment. In previous reports, the ORR for ibrutinib-based regimens

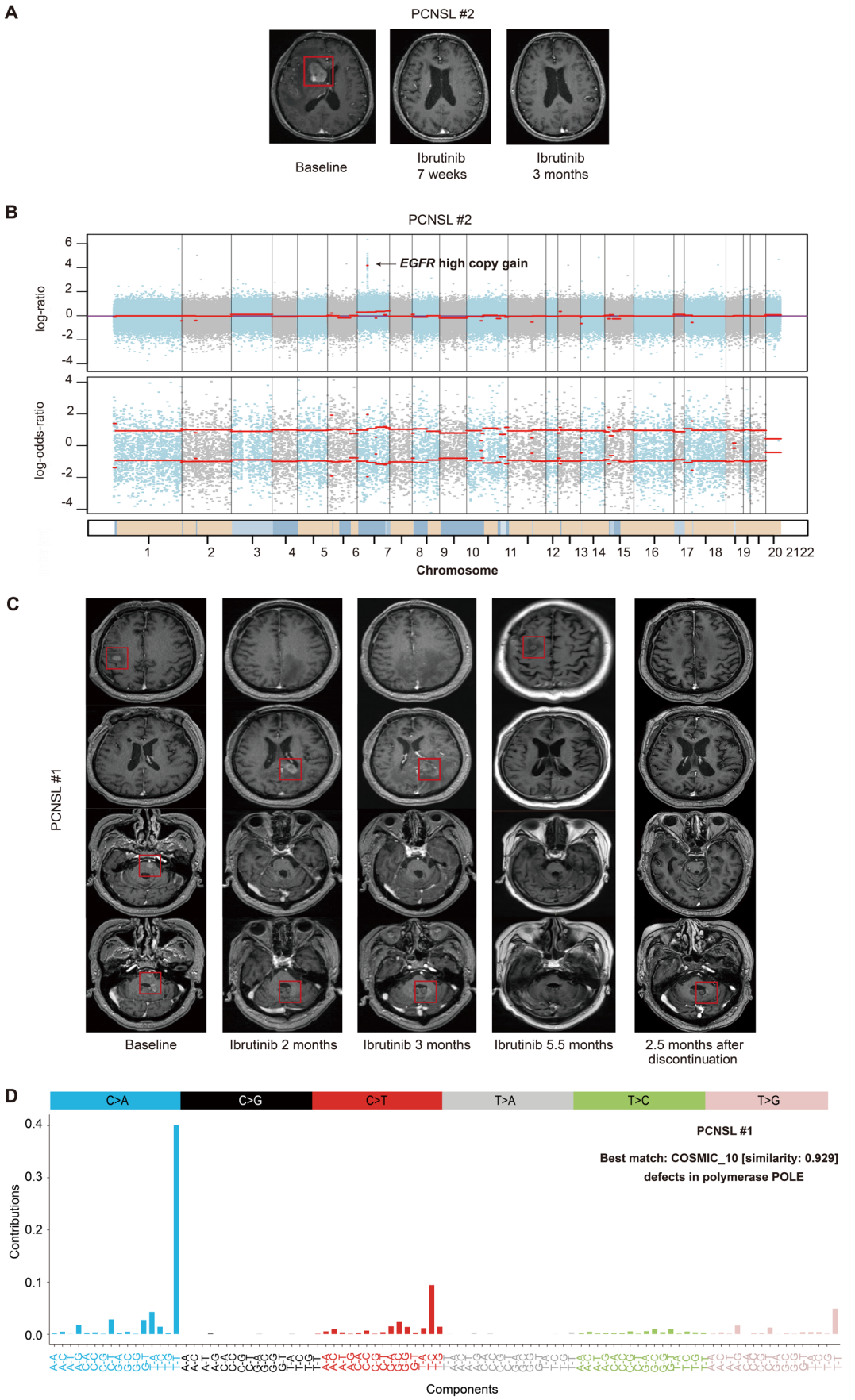

Fig. 2. Response to ibrutinib and genetic events in 2 primary central nervous system lymphoma (PCNSL) patients. A. Response to ibrutinib treatment was assessed using magnetic resonance imaging (MRI) in PCNSL patient #2; B. Log ratio of each chromosome of PCNSL #2. Chromosome numbers are labeled in chromosome cytoband at the bottom. Arrows indicate the location of epidermal growth factor receptor (EGFR). Tumor was assessed by enhanced MRI and is marked with red diamonds; C. Dynamic changes in the tumor lesion at different time points along with ibrutinib treatment in PCNSL #1; D. Mutational signature of PCNSL #1 according to 96 trinucleotide mutational patterns. The similarities with the COSMIC-defined signature are labeled. Base substitutions are labeled in different colors. Tumor was assessed using enhanced MRI and is marked with red diamonds. The MRI images obtained at different time points and at the same scanned level during ibrutinib therapy were piled together, including the centrum semiovale, periventricular, mesencephalon, and fourth ventricle from the top to bottom

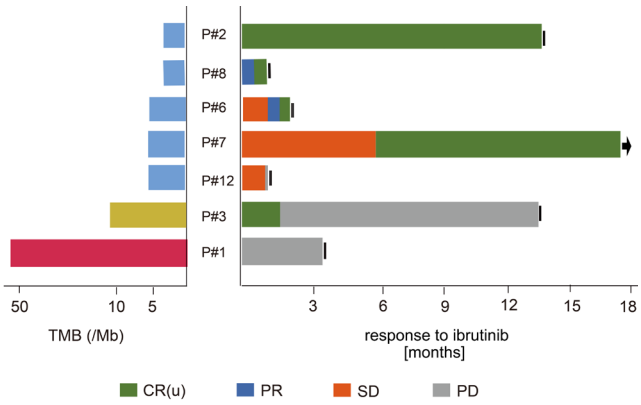

Fig. 3. Relationship between response to ibrutinib and tumor mutation burden (TMB). The height of each bar in the upper panel represents TMB (/Mb) and in the lower panel represents the response to ibrutinib (months) in 7 primary central nervous system lymphoma (PCNSL) patients with whole-exome sequencing (WES) data. Barcodes of samples are labeled at bottom. The distinct colors of the bars in the upper panel represent different levels of TMB. Different colors in the lower panel represent various disease stages. The symbols represent different ibrutinib usages: ongoing treatment (⟶); discontinued (❙). In the left panel, TMB is illustrated by bars with different lengths and colors (red indicates TMB > 50/Mb, yellow indicates TMB > 10/Mb, blue indicates TMB < 10/Mb)

CR – complete remission; CR(u) – complete remission unconfirmed; PR – partial remission; SD – stable disease; PD – progressive disease.

in r/r PCNSL ranged from 45% to 89%, and for ibrutinib monotherapy from 50% to 59%.[6–8,27–30] Consistent with previous reports, the current response rate for the PCNSL cohort (75%) confirmed the effectiveness of ibrutinib for r/r cases in a real-world clinical setting. However, no relationship was found between the responses to ibrutinib and previous regimens, possibly due to the limited sample size.

Although encouraging remission was achieved by ibrutinib, the PD after remission revealed in this study (45%) and in prior reports (25–71%) indicates persisting challenges.[7–9] The newest regimens that have been explored in B-cell malignancies, such as combining a BTK inhibitor with CAR-T immunology therapy, may provide a promising potential for targeted therapy resistance patients.[31]

In line with previous reports, the safety profile of ibrutinib-based regimens was confirmed to be of low toxicity to organs.[7,9,32] However, incidences of infection and the need to discontinue ibrutinib were observed in nearly half of the cohort, consistent with previous reports,[6,8] indicating significant problems during the therapy process. A previous study suggests that the inhibition of BTK or interleukin 2-inducible T-cell kinase (ITK) can result in immune suppression, especially when corticosteroids are applied.[33,34] According to the findings by Lionakis et al., in clinical cases and murine models, ibrutinib as a BTK inhibitor might also block macrophage activation in the immune system during, especially fungal, infections.[6] Hence, studies targeting optimized regimens and infection prevention during ibrutinib therapy are needed.

Based on our limited patient sample, a preliminary relationship between genomic alternations and the response to BTK inhibitors was indicated, which requires future validation. The effects of ibrutinib were found to be independent of mutations in *MYD88*, *PIM1*, *CD79B*, and in the BCR or NF-κB pathways, which is in accordance with findings by Grommes et al. and Soussain et al. stating that the ibrutinib response would not be impacted by mutation.[7,8] Wilson et al. indicated that both MCD and N1 subtypes could respond to ibrutinib-based regimens.[13] However, in this study, we observed that 1 MCD case resisted ibrutinib due to complex genomic alterations (PCNSL #1). This case harbored highly complex genomic alterations and showed multiple recurrent intracranial tumors as well as multiresistance to therapeutic regimens, suggesting that the genomic features of ultra-hypermutations and mismatch repair deficiency may contribute to the efficacy of ibrutinib more than molecular classification.[35]

In contrast, another unclassified patient under the LymphGen system with low TMB and high copy gains of *EGFR* responded quickly to ibrutinib, and his CR status was maintained for more than 2 years. Even though the off-target effects of ibrutinib on *EGFR* and *ITK* are recognized as disadvantages in clinical applications,[36,37] they might have played a role in suppressing the overexpression of *EGFR* to control the tumor in this patient. Although more cases and explorations are warranted to confirm this association, all of the above findings indicate that comprehensive genetic factors need to be considered for BTK inhibitor effects.

Overall, in this retrospective single-center study, we summarized the clinical benefits, safety profile and the impact of genetic variants on ibrutinib-based regimens in r/r CNSL patients in a clinical setting.

Limitations

The empirical results reported here should be considered in the light of some limitations. As only 2 SCNSL patients were included in this study, the current results may deviate from real-world practice. More SCNSL cases need to be evaluated in future studies.[7,27,28] The influences of genetic variants on ibrutinib therapies were examined in only 7 patients, thus the negative influences of genetic complexity need to be validated and further examined in a larger cohort. Additionally, as a retrospective study, heterogeneity in treatment regimens was unavoidable. Hence, a well-designed prospective study is needed in the future.

Conclusions

In this study, we verified the low organ toxicity of ibrutinib-based regimens, and expounded the relationship between genomic alterations and responses to a BTK inhibitor. High genomic complexity (especially TMB) may negatively impact responses toward ibrutinib, suggesting that patients harboring less genetic events may benefit more from an ibrutinib regimen.

Our results are consistent with previous studies, indicating low organ toxicity and a high effectiveness for ibrutinib in r/r cases. Our results cast a new light on the response to ibrutinib-based regimens in patients possessing a low genomic complexity.

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