Zanubrutinib plus Ixazomib and Dexamethasone in newly diagnosed symptomatic Waldenström macroglobulinemia:a phase II study

Short title: ZID for Waldenström macroglobulinemia

Wenjie Xiong[1,2*], Yuting Yan[1,2*],Tingyu Wang[1,2*], Weiwei Sui[1,2*], Ying Yu[1,2], Tengteng Yu[1,2], Rui Lyu[1,2], Yi Wang[1,2], Wei Liu[1,2], Huimin Liu[1,2], Gang An[1,2], Yan Xu[1,2], Wenyang Huang[1,2], Dehui Zou[1,2], Lugui Qiu[1,2#], Shuhua Yi[1,2#]

[1]State Key Laboratory of Experimental Hematology, National Clinical Research Center for Blood Diseases, Haihe Laboratory of Cell Ecosystem, Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin 300020, China

[2]Tianjin Institutes of Health Science, Tianjin 301600, China

[#]**Correspondence:**

Dr. Lugui Qiu, MD

[1]State Key Laboratory of Experimental Hematology, National Clinical Research Center for Blood Diseases, Haihe Laboratory of Cell Ecosystem, Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin 300020, China

[2]Tianjin Institutes of Health Science, Tianjin 301600, China

No. 288, Nanjing Road, Tianjin 300020, China

E-mail: qiulg@ihcams.ac.cn

Telephone number: 86-13821266636

Dr. Shuhua Yi, MD

[1]State Key Laboratory of Experimental Hematology, National Clinical Research Center for Blood Diseases, Haihe Laboratory of Cell Ecosystem, Institute of

Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin 300020, China
[2]Tianjin Institutes of Health Science, Tianjin 301600, China
No. 288, Nanjing Road, Tianjin 300020, China
E-mail: yishuhua@ihcams.ac.cn
Telephone number: 86-15900265415
*WX, TY, TW, and WS contributed equally to this work
#SY and LQ contributed equally to this work

Text word count: 4476/4000
Abstract word count: 249/250
Number of figures and tables: 6
Number of references: 28
Scientific category: Lymphoid Neoplasia

Translational Relevance:

Waldenström macroglobulinemia (WM) is a rare type of lymphoma, with no optimal treatment. Although BTK and proteasome inhibitors have shown promising outcomes, achieving deep remission remains challenging. This study presents the first investigation into a time-limited regimen combining zanubrutinib, ixazomib, and dexamethasone (ZID) for newly diagnosed symptomatic WM patients. The ZID regimen offers promising deep remission rates and good tolerance, providing a potential new therapeutic approach for this disease.

Conflict of interest Statement

We declare no competing interests.

Abstract

Purpose: Waldenström macroglobulinemia (WM) is a rare type of lymphoma, with no optimal treatment. BTK inhibitor have shown promising outcomes, yet achieving deep remission (VGPR or CR) remains challenging and time-limited therapy with proteasome-inhibition has not been reported. We conducted a phase 2 clinical trial (NCT04463953) to evaluate the efficacy and safety of combining zanubrutinib, ixazomib and dexamethasone (ZID) in newly diagnosed WM patients.

Patients and Methods: 27 patients were enrolled in the study. Patients received ZID induction therapy for up to six 28-days' cycles, followed by consolidation therapy up to total 24 cycles. The primary endpoint was the deep remission rate.

Results: Overall, 24 of 27 enrolled patients completed induction treatment. One patient (4.2%) achieved CR. 10 patients (41.6%) achieved VGPR. The overall, major and deep remission rates were 100%, 95.8% and 45.8%, respectively. The median time to response was 2 months (range, 1-5). Five of 22 patients had *CXCR4* mutation, with no disparity in the deep remission between the patients with/without *CXCR4* mutation (40% vs 50%, P=0.594). The median abnormal lymphocyte (7.6% vs 1.6%, P =0.0019) and plasma cells (0.28% to 0.02%, P =0.0306) in bone marrow were significantly reduced after treatment. With a median follow-up of 30.9 months (range, 15-42). The estimated median PFS and OS were 40 months (95% CI:35.5-44.5) and not reached, respectively, with no difference in patients with/without *CXCR4* mutations. The most common AE was hematological toxicity.

Conclusion: ZID regimen might offer deep remission and provided a time-limited BTKi therarpy in WM patients.

Trial Registration

The study has been registered at clinicalTrails.gov (NCT04463953)

Keywords

82 Zanubrutinib, Ixazomib, Dexamethasone, Waldenström macroglobulinemia.

83

Introduction

Waldenström macroglobulinemia (WM) is a B-cell Non-Hodgkin lymphoma, characterized by the infiltration of monoclonal B lymphocytes in the bone marrow, accompanied by monoclonal IgM production.[1,2] WM is associated with genetic mutations observed in the MYD88 and CXCR4 genes.[3] *MYD88*^{L265P} mutation is an important pathogenesis factor of WM, found in 95-97% of patients[2], leading to the recruitment of Bruton's tyrosine kinase (BTK) protein and activation of the downstream NF-kB signaling pathway.[4] Over the last two decades, there has been substantial growth in the treatment options for WM. Current guidelines recommend BTK inhibitors (BTKi) such as zanubrutinib alone or ibrutinib combined with rituximab, rituximab or proteasome inhibitor (PI)-based immunochemotherapy such as bortezomib combined with rituximab and dexamethasone (BDR), bendamustine and rituximab (BR), and rituximab combined with cyclophosphamide and dexamethasone as the first-line treatment for WM. [2,5-8]Moreover, BTKi have shown high efficacy in treating WM, with an overall response rate of 90-100%[9,10]. However, BTKi monotherapy results in a low rate of very good partial remission (VGPR) (20%-30%), and few complete remission (CR) [11,12] . In addition, it also has demonstrated lower efficacy in patients without the MYD88^{L265P} mutation[13]. And *CXCR4* mutations adversely impacted the response and survival outcomes of BTKi in WM patients.[14] Furthermore, ibrutinib monotherapy also necessitates long-term drug administration, posing challenges due to its high cost and propensity for developing drug resistance and intolerable toxicities. Premature discontinuation of the medication may also result in rapid IgM rebound. [15,16]

Studies have demonstrated that the combination of ixazomib with rituximab and dexamethasone (IRD) is effective in treating WM, with a overall response rate of 96% and VGPR rate of 15%[17]. However, the deep response rate (CR orVGPR) is 19%. Moreover, the depth of response to IRD has been adversely affected by *CXCR4*

mutations, in addition to prolonging the time to response and major response. [17] Co-administration of BTKi with ixazomib has been shown to enhance the tumor-killing effect.[18] Compared to first-generation BTKi, zanubrutinib's enhanced pharmacodynamic characteristics have resulted in sustained responses and better tolerability. The minimal rate of discontinuation indicates a favorable safety profile, making zanubrutinib well-suited for long-term use in combination with PIs.[19] Moreover, zanubrutinib has achieved a high ORR and low AEs and is one of the therapeutic agents recommended in the current NCCN guidelines. Despite the high ORR for patients on different treatment regimens, the deep remission rate is still less than 30%[11].

Enhancing the depth of response in patients with WM remains a pressing clinical imperative. Moreover, there is a need to find a safer approach for the long-term use of BTKi and to lessen the treatment duration and economic burden. This study aims to investigate the treatment efficacy and safety profile of the zanubrutinib plus ixazomib and dexamethasone (ZID) regimen in newly diagnosed patients with WM and explore the deep response rate.

Patients and methods

Study design and participants

This single-center, phase 2 clinical trial included patients with newly diagnosed symptomatic WM. Patients were eligible if they received a centrally confirmed diagnosis of WM that required treatment according to the previously reported criteria[1]. All patients were newly diagnosed, ≥18 years old, and met the recommendations for treatment according to the Second International Workshop on WM. Additional inclusion criteria were an Eastern Cooperative Oncology Group performance status (ECOG-PS) of 0–2 and a life expectancy of ≥6 months. Patients with serious complications, liver or renal lesions unrelated to lymphoma, or clinical nervous

dysfunction were excluded. The representativeness of study participants showed in the **supplemental Table 1**. Written informed consent was obtained from each subject or each subject's guardian.

The trial complied with the ethical principles outlined in the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice Guidelines and patients provided informed consent. The protocol was reviewed and approved by our institutional review board (BDH-WM2020/04) and registered at ClinicalTrials.gov (NCT04463953). This study protocol was designed in the accordance with the SPIRIT guidelines and the results are reported following the CONSORT recommendations for non-randomized interventional trials.

Procedures

In the induction phase, eligible patients received 6 courses of ZID treatment, and the efficacy was evaluated after every 2 courses. ZID treatment consisted of zanubrutinib 160 mg twice daily, orally, ixazomib 4 mg on days 1, 8, and 15, and dexamethasone 20 mg on days 1, 8, and 15, one course of treatment was given every 4 weeks. If a minimal response (MR) or above occurred, the original regimen was continued. If there was no treatment response (disease stabilization or progression) after the initial 4 courses of treatment, the patient was withdrawn from this study. After 6 courses they entered the maintenance phase, where zanubrutinib 160 mg was administered orally twice a day during the whole treatment period and the ixazomib combined with dexamethasone (ID) regimen was administered once every 3 months. Zanubrutinib was stopped in the last month, and treatment ended with the ID regimen to prevent IgM rebound.[15,16]Treatment would be stopped if maximum response was achieved after the induction therapy or up to 24 months. The study protocol is shown in **supplemental Figure 1**. The inclusion/exclusion criteria information showed in **supplemental Data 1**.

Efficacy and safety assessments were performed every cycle by laboratory assessment and physical examination during induction and maintenance treatment. Bone marrow biopsy (BMB) and flow cytometry was used to evaluate cellular components. Minimal residual disease (MRD) was assessed using eight-color flow cytometry. An MRD negative result was defined as a clonal malignant cell count of <10−4 (0.01%). The antibody markers included CD3 (RRID:AB_10611584), CD5 (RRID:AB_2737986) , CD10 (RRID:AB_2737879), CD19 (RRID:AB_400151), CD20 (RRID:AB_11153856), CD23 (RRID:AB_2740144), CD25 (RRID:AB_2034002), CD27 (RRID:AB_1645474), CD28 (RRID:AB_2744355), CD38 (RRID:AB_2740093), CD56 (RRID:AB_1645578), CD79b (RRID:AB_2739165), CD81 (RRID:AB_2739768), CD117 (RRID:AB_400563), CD200 (RRID:AB_2738099), CD138 (RRID:AB_393889), FMC7 (RRID:AB_400174), Light chain kappa (RRID:AB_11180435) or Light chain lambda (RRID:AB_10583336). MRD was detected at every 3 months during induction and then every 6 months during treatment. [20]If a patient had extramedullary lesions, CT scans were performed every 3 months during induction and every 6 months after treatment. AEs were assessed according to the National Cancer Institute's Common Toxicity Criteria version 4.03. Genetic mutation analysis was detected by the target sequencing. The target sequencing and droplet digital PCR (ddPCR) methods were used to detect MYD88 ^{L265P} mutations in patients. Mutation was detected by either method was defined as mutation and target sequencing were used to detect CXCR4 mutations in patients.[21]

The chromosome karyotypes and interphase FISH analysis was performed on bone marrow samples at diagnosis. Bone marrow cells were cultured for 24 hours (h) without immunostimulatory factor. The FISH probe panel included the loci centromere 12 (CEP12), 13q14.3 (LSI D13S25/RB-1), 17p13 (LSI TP53), 11q22 (LSI ATM) and 6q21(MYB).

Endpoints and assessments

The primary endpoint was the deep response rate (≥VGPR rate) after 6 courses of the ZID regimen. The key secondary endpoints were safety, objective response rate (ORR), major response rate (MRR), CR, VGPR, partial response (PR), and minimal response (MR). Responses were based on modified consensus criteria from the sixth International Workshop on WM.[12,22] The response criteria are shown in the supplementary materials (**Supplemental Table 2**). Patients who obtained VGPR or CR were classified as a deep response. The overall response was defined as the proportion of patients who achieved a minor response, PR, VGPR, or CR. A major response was defined as the proportion of patients with a PR, VGPR, or CR. The response assessments were based on an intent to treat analysis and all the 24 patients who received treatment were included. The IgM rebound was defined as a 25% increase in serum IgM level, with an absolute increase of at least 500 mg/dL, following discontinuation of zanubrutinib.

Other endpoints included progression-free survival (PFS), duration of response (DOR), and overall survival (OS). PFS was the time from the treatment until disease progression (except only IgM elevation) or death from any cause. IgM rebound related to therapy will not be considered as progressive disease[1]. DOR was the time from the initial qualifying response, including minor response, PR, VGPR, and CR, until progression (except only IgM elevation) or death. OS was the time from treatment to death due to any cause. Patients who were lost to follow-up or still alive at the end of the study were censored at the last known date alive.

The safety endpoints were the incidence and severity of hematological and non-hematological AEs. The incidence, outcome, and severity of AEs were assessed, including AEs of interest (AEIs).

Immune tumour microenvironment

Flow cytometry (FCM) was used to conduct a multiparametric analysis of the lymphocyte subsets of the patients to analyze the changes in the immune microenvironment before and after ZID treatment. The data acquisition and subsequent analysis were performed on BD FACSCanto™ system (RRID:SCR_018056) and FACSDiva™ software (RRID:SCR_000055), respectively.

Statistical analysis

Studies have shown that the VGPR rate of the IRD regimen for the treatment of newly diagnosed WM is 15%.[17]This study hypothesizes that the ZID regimen has a 15%-20% higher VGPR rate than that of the ixazomib-based regimen, reaching 45% or above. If VGPR rate $\geq$ 45%, this study estimated that an interim test (Group Sequential test) required a total sample size of 57 patients to achieve a false positive rate of α=0.05, a false negative rate of β=0.2 and a superiority difference of 15% higher than the historical. Including a 10% dropout rate in each group, 63 patients were required. An interim analysis was conducted number of patients reached 32.

However, during the early stages of enrollment, China was still experiencing the effects of COVID-19, which led to logistical challenges and slowed the recruitment process. Additonally, since ixazomib was not covered by health insurance, participants faced increased financial burdens. Lastly, considering that the patients' deep remission rate is high but they cannot discontinue the drug safely with high IgM rebound, we terminated the recruitment early. Eventually, 27 patients were enrolled.

We used the Kaplan-Meier method to estimate the survival curves for PFS, DOR, and OS and used a log-rank test to assess the potential differences between groups. The hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated using the Cox proportional hazards regression model with a two-sided level of 0.05. Descriptive statistics was used to summarize the data of the efficacy and safety analysis.

All statistical tests were two-sided, and *P* values less than 0.05 were considered to be statistically significant. Statistical analyses were performed using the Statistical

Package for the Social Sciences version 25.0 (IBM Corporation, Armonk, NY, USA, RRID:SCR_002865), GraphPad Prism version 7.0 (GraphPad Software, Inc., San Diego, CA, USA, RRID:SCR_002798), and R version 4.0.5.

Data availability

Data are available from the corresponding author on reasonable request. We encourage researchers interested in data sharing and collaboration to contact the corresponding author. Data are not publicly available to protect patient privacy.

Results

Patient characteristics

Between July 2020 and August 2022, 27 patients were assessed for eligibility, of which three patients were excluded from this study. One due to cerebral hemorrhage, and 2 withdrew for personal reasons. Eventually, 24 patients completed the induction therapy and were included in the analysis (**supplemental Figure 2**). The baseline characteristics are presented in **Table 1**. The median age was 62 years (range: 32-73), with 9 patients (37.5%) being 65 years of age or older. The median baseline hemoglobin level was 87 g/L (range: 68–127), and the median baseline IgM was 41.9 g/L (range: 6.4–125). The proportion of patients in the low, intermediate, and high-risk International Prognostic Scoring System for WM (ISSWM) group was 16.7%, 58.3%, and 25%, respectively. A total of 23 patients (95.8%) had *MYD88* ^{L265P}mutation. Genetic mutation analysis by target sequencing was conducted on 22/24 patients. The most prevalent mutations were *MYD88* ^{L265P}*(100%), CXCR4 (22.7%), KMT2D (13.6%), IGLL5 (13.6%).* Five patients had CXCR5 mutation. Three of the five patients had CXCR4 p.S338* mutation, one patient had CXCR4 p.S341Pfs*25 mutation and one had CXCR4 p.T318Nfs*26 mutation. Of the 24 patients, 21 underwent successful *IGHV* mutation detection, revealing that 19 patients had *IGHV* mutation (90.5%) using 2% difference from the geamline as the cutoff. The

most common IGHV fragments were IGHV3-23 (35%), followed by IGHV3-30 (10%) (**supplemental Figure 3A-B**).

All patients had chromosome karyotype detected and 8 patients had abnormal karyotype. Most patients had chromosomal deletions or amplifications. The most common chromosomal deletions were sex chromosome deletions and 6q-. The results of chromosome karyotype were showed in the **supplemental Table 3**. All the patients had FISH detected and 7 patients had 6q deletion (29.2%).

Response

All 24 patients completed the induction phase, with the median duration of the ZID regimen being 16.0 months (range: 6.0-24.0). After treatment, 1 patient (4.2%) achieved CR, 10 patients (41.6%) achieved VGPR, 12 patients (50%) attained PR, and 1 patient attained MR (4.2%). The MRR was 95.8% (95%CI: 87.2-104.5), with a deep response rate (VGPR or CR) of 45.8% (95%CI: 24.3-67.3). Notably, among the 5 patients with *CXCR4* mutation, 3 (60%) achieved PR and 2 (40%) achieved VGPR. The deep response rate in patients with *CXCR4* mutation compared to those without was 40% vs 50% (*P*=0.594), respectively (**Figure 1**).

The median time to response (TTR) was 2 months (range: 1-6), median time to best response (TTBR) was 5 months (range: 1-15), and the median time to VGPR (TTVGPR) was 6 months (range: 2-14). In patients with *CXCR4* mutation, the median TTR , time to MRR (TTMR) and TTBR were 2 months (range: 1-3), 2 months (range 1-5) and 4 months (range: 1-5) respectively, which was similar to those without this mutation [TTR 2 months (range:1-6) , *P*=0.533; TTMR 2 months (range 1-11), *P*=0.512; TTBR 5 months (range 2-15), *P*=0.207]. Two patients with CXCR4 mutation achieved VGPR and the TTVGPR were 5 and 8 months, respectively. In patients without CXCR4 mutation, the TTVGPR were 5 months (range 2-15), which was also comparable to those with CXCR4 (*P*=0.774).

The factor that was associated with deep response rate was serum IgM level <70 g/L (*P*=0.009). No other evaluated factors were associated with deeper remission (*P*> 0.05 for all variables), such as age > 65 years, B2M level > 3 g/L, hemoglobin level < 115 g/L, platelet<100×10^9/L, with B symptom, presence of hepatomegaly, splenomegaly or lymphadenopathy, elevated LDH, *MYD88*, and *CXCR4* mutational status, cytogenetic abnormality, International Prognostic Scoring System for WM (IPSSWM) score, revised IPSSWM score (**supplemental Table 4**).

Tumor burden and Minimal residual disease

Overall, at best response, the median serum IgM level decreased from 41.9 (range: 6.4–125) g/L to 4.3 (range: 0.93–86.0) mg/dL and the median hemoglobin level increased from 87 (range: 68–127) g/L to 142 (range: 91–175) g/L (*P*<0·001) (**Figure 2A-B**). Seven patients had lymph node enlargement. The lymph node response rate was 100%, and the median SPD dropped from 10.6 to 6.04 cm^2 (*P*=0.0119, **Figure 2C).** Of note, 51.7% of patients had a decrease in SPD of 50% or more.

The median tumor cell percentage of BM by FCM decreased from 7.8 % (range: 0.6-60.1) to 1.9 % (0-9.3) (*P*=0.0008). The median tumor cell percentage of BM by bone marrow biopsy (BMB) decreased from 50% (range: 5-90) to 5% (range: 0-60), *P*<0.0001(**Figure 2D-E**). Cellular components analysis revealed that the ZID regimen could eliminate abnormal lymphocytes as well as abnormal plasma cells in the bone marrow. By FCM, the median abnormal lymphocyte reduced from 7.6% (range: 0.5-60.1) to 1.6% (range: 0-9.25) (*P* =0.0019), and the median abnormal plasma cells were reduced from 0.28% (range: 0-1.39) to 0.02% (0-0.54) (*P* =0.0306) (**Figure 2F).** After treatment one patient achieved MRD negative.

For the patients with *CXCR4* mutation, the tumor burden detected by FCM before treatment was higher than that of the patients without *CXCR4* mutation (43.2% vs 6.6%, *P* =0.0016). The tumor burden also decreased rapidly in the *CXCR4* mutated

patients. The median tumor cell of BM by FCM reduced from 43.2% (range: 3.4-60.1) to 3.3% (range: 0.2-9.3) (*P*=0.0515).

Immune tumour microenvironment

Peripheral blood lymphocyte subsets were tested in 21 of 24 patients. The level of IgM was positively associated with the percentage of NK cells (R=0.54, *P*=0.0096) and negatively associated with the percentage of CD3+ T cells (R=-0.49, *P*=0.021) and CD4+ helper T cells (R=-0.63 *P*=0.0023) (**Figure 3A-C**). There was no significant correlation between the proportion of IgM and CD8+ cytotoxic T cells (*P*=0.21), Treg cells (*P*=0.76) (**supplemental Figure 4A-B**). Patients who achieved a deep response rate had a higher proportion of CD3+ T cells (*P*=0.0317) **(supplemental Figure 4C)** and CD4+ helper T cells. The Median proportion of CD4 was 46.5% in the patients who achieved deep remission which was significantyly higher than the patients without deep remission. (46.5% vs 30%, *P*=0.0039) (**Figure 3D**). Other lymphocyte subsets such as CD8+ cytotoxic T cells, NK cells, and Treg had no significant difference in the response rate (**supplemental Figure 4D-F**). Patients achieved partial restoration of immune function, had a significant increase in the proportion of CD4 helper T cells (median CD4 helper T cell before treatment vs after treatment: 39% vs 48%, *P*=0.0115), CD8 cytotoxic T cells (median CD8 cytotoxic T cell before treatment vs after treatment: 18% vs 25%, *P*=0.0252) (**Figure 3E-F**). Other TME cells also recovered immune function after treatment, but the difference was not statistically significant (**supplemental Figure 4G-I**).

Survival

With a median follow-up of 30.9 months (range: 15.0-40.5), five patients progressed, and the estimated median PFS was 40.0 months (95% CI: 35.5-44.5) (**Figure 4A**). Only one patient who achieved deep remission progressed. The estimated median PFS for patients who achieved deep remission was not reached (NR), which was slightly higher than those without deep remission (NR vs. 37.0 months, respectively, 95% CI

28.1-45.8, *P*=0.283) (**supplemental Figure 5A**). No significant difference in OS was observed between the two groups (**supplemental Figure 5B**). The factor associated with better PFS was normal chromosome karyotype (*P*=0.014) . *CXCR4* mutational status, IPSSWM score, and revised IPSSWM score (*P*> 0.05 for all variables) were not associated with adverse PFS (**supplemental Table 5**). Two patients died at the time of follow-up (one because of COVID-19 infection at 30.5 months and another due to pulmonary infection at 20 months). The median OS was NR (**Figure 4B**).

Importantly, the median PFS in patients with CXCR4 mutation was 32.1 months, which was similar with that without this mutation (32.1 vs 40 months, *P*=0.554, HR 1.860, 95%CI: 0.123-28.02). Furthermore, no significant difference in OS was observed between the two groups (*CXCR4* mutation vs *CXCR4* WT: 32.1 vs NR, *P*=0.247, HR: 4.45, 95%CI: 0.124-160.1) (**Figure 4C-D**).

Safety

The AEs are shown in **Table 2**. The most common hematological AEs were neutropenia (33.3%), thrombocytopenia (29.2%), and leukopenia (16.7%). The most common non-hematological AEs were insomnia (20.8%), fever (20.8%), rash (16.7%), infection (16.7%), peripheral neuropathy (16.7%), and fatigue (16.7%). Grade 3 or higher AEs included infection (12.5%), thrombocytopenia (8.3%), insomnia (4.2%), hyperglycemia (4.2%), pruritus (4.2%), and hyperglycemia (4.2%). One patient was hospitalized with appendicitis after stopping the drugs. Two patients had COVID-19 infection and one patient died after developing a COVID-19 infection at 30.5 months of medication which was not related to treatment. All patients in this study had been vaccinated. No patient had fungal infection. Ixazomib was reduced to 3 mg once a week in 2 patients due to insomnia and the other 2 patients briefly discontinued ixazomib due to thrombocytopenia. Three patients had reduced zanubrutinib dose due to rash, the other 3 patients discontinued treatment due to grade 3-4 infection (COVID19, pulmonary infection or acute appendicitis). Dexamethasone was

discontinued in one patient due to intolerable hyperglycemia. During the maintenance phase, four patients did not proceed with the maintenance treatment. One patient experienced disease progression after 17 months of medication and was subsequently shifted to receive the BR regimen; One patient was withdrawn from the study after 8 months of medication due to intolerable itching; One patient died after developing a COVID-19 infection at 30.5 months of medication; Lastly, one patient withdrew from the study for personal reasons and declined further follow-up at the 12-month mark.

IgM rebound and disease progress after drug discontinuation

At follow-up, 14 patients completed their treatment regimen. Two of these patients deviated from the cessation protocol, opting to persist with zanubrutinib therapy. The remaining 12 patients terminated their treatment. The median duration from the cessation of the drug to the last follow-up was 5 months (range: 1-16). A notable 75% (9/12) of the patients experienced an IgM rebound, occurring at a median interval of 2 weeks (range 2-4 weeks). Among the 9 patients, three patients resumed zanubrutinib by themself due to elevated IgM levels, in the absence of physician's guidance. One patient died from COVID-19 infection 6 months after the discontinuation. The other 5 patients continue to be monitored for IgM rebound, exhibiting no clinical symptoms, over a median observation period of 6 months (range: 4-16). Among the 3 patients who resumed the zanubrutinib treatment, 2 patients who achieved PR encountered disease progression after 11 and 14 months, respectively, while 1 sustained VGPR. **Supplemental Table 6** shows the outcomes and disease status of patients who completed the treatment. **Supplemental Figure 6** showed the swim lane plots of efficacy in patients treated with ZID.

Discussion

This was the first study to explore the time-limited treatment of BTKi zanubrutinib combined regimen in newly diagnosed patients with WM. The study revealed that

ZID had a high deep remission of 45.8% (95% CI: 24.3%-67.3%) and good tolerance, thereby achieving the study's primary goal. Over a median period of 6 months, patients who discontinued treatment showed no additional clinical symptoms apart from IgM rebound, underscoring that the ZID regimen is a optional choice for WM patients who want time-limited BTKi therapy.

Clinical investigations have indicated that the deep response rates in patients with WM undergoing treatment with either ibrutinib or zanubrutinib monotherapy, or in combination with immunochemotherapy, remain suboptimal. Zanubrutinib has achieved a high ORR and low AEs and is one of the therapeutic agents recommended in the current NCCN guidelines. [7,12] However, the deep remission rate is still less than 30%. Our study showed that the ORR was 100%, which was similar to the IRD regimen[17] and zanubrutinib monotherapy[12], but, the patients' deep remission rate was significantly improved and was substantially higher than the IRD and zanubrutinib monotherapy regimens.

For patients with *MYD88L265P* and *CXCR4* mutated, the ibrutnib monotherapy is not effective. The ASPEN study showed that compared to ibrutinib, zanubrutinib further improves the deep remission rate. Zanubrutinib also proved to be efficacious in $MYD88^{L265P}$ and *CXCR4* mutated patients. [12] Our study reported that of the 5 patients with *CXCR4* mutation, 3 patients achieved PR and 2 patient achieved VGPR after treatment of ZID regimen. The deep response rate in patients with *CXCR4* mutation was similar with the *CXCR4* wide-type patients, proving that the ZID regimen improved the efficacy of patients with *CXCR4* and *MYD88* mutations and that patients regardless of mutation status can benefit from this treatment. In addition, similar to IRD[17] and zanubrutinib monotherapy[12], ZID regimen can also achieve a rapid response to treatment. The median time to response and best response in this study were 2 months and 5 months.

Recently, a phase II study by Parrondo et al evaluated time-limited (24 cycles) treatment with ibrutinib and ixazomib combination in WM. Though 28.5% discontinued treatment due to toxicity, a clinically significant ORR and PFS of 76.2% and 22.9 months were attained with no patient achieving a CR.[23] In our study, all patients completed the induction phase with a median duration of 16 months (range: 6-24) with a deep response rate of 45.8% (95% CI: 24.3-67.3). At a median follow-up of 30.9 months, the median PFS of the ZID regimen in the present study was greater than the ibrutinib and ixazomib combination at 40.0 months. The ASPEN[12] study reported a favourable PFS outcome in patients treated with continuous zanubrutinib. Moreover, the INNOVATE[24] study reported a longer PFS in the ibrutinib-rituximab cohort compared with the placebo-rituximab cohort[24]. Bendamustine combined with rituximab was also a time-limited treatment in WM with a reported a longer disease control period than rituximab combined with cyclophosphamide, doxorubicin, vincristine, and prednisone with a PFS ranging from 65.0-69.5 months[25,26]. However due to the smaller sample size and a shorter-follow-up period, cross-trial comparison was not feasible.

TME is considered essential to maintain tumor cell growth and survival. Recent study had shown a strong relationship between the lymphocyte subsets of TEM in patients with waldenstrom and prognosis[27]. And ibrutinib can modulated the immunosuppressive microenvironment.[28] This study also demonstrated that the ZID regimen effectively reduces the tumor burden, removes IgM, and improves the immune TME. Our analysis showed that immune TME dysfunction exists in WM patients and is associated with IgM levels and deep response. Treated with ZID regimen could significantly improve the immune TME function and increased the deep remission.

IgM rebound is an important influence on ibrutinib discontinuation, with up to 40-70% of patients experiencing IgM rebound after discontinuing ibrutinib therapy [15,16] can

potentiate IgM-related morbidity, such as hyperviscosity, PN, etc.[16] Studies on WM patients treated with ibrutinib have reported the acute onset of symptomatic hyperviscosity in 16% of patients with an IgM rebound.[15] Commonly employed treatments such as time-bound BR appear to provide longer disease control with similar safety profiles while avoiding the risk of IgM rebound. Of note, in our study, when combined with ixazomib and dexamethasone, 25% of patients did not exhibit IgM rebound. For patients in the presence of IgM rebound, there were still no other clinical symptoms at a median of 6 months of drug discontinuation.

Our study also explored the selection of personalized treatment for WM by investigating the factors influencing the withdrawal time of patients upon drug discontinuation with continuous follow-up. Doses of zanubrutinib and ixazomib were reduced due to rash and insomnia, respectively, whereas, infection, thrombocytopenia, and intolerable hyperglycemia were the primary causes of discontinuation of zanubrutinib, ixazomib, and dexamethasone, respectively. The maintenance treatment was discontinued in four patients due to disease progression, intolerable itching, COVID-19 infection, and personal reasons (1 patient each). These findings provide insight to deal with treatment discontinuation and to better manage these patients in the future. When compared to zanubrutinib monotherapy or IRD regimen, the ZID regimen provides a high depth of response rate with a limited number of treatment cycles to achieve the study endpoint.

However, this study has some limitations. Our study included a small sample size with a short duration follow-up and only a few patients had CXCR4 mutations. Therefore, the results need to be warranted in studies with a larger sample size. Secondly, This study encountered significant challenges in patient enrollment due to its timing during the COVID-19 pandemic. Additonally, since ixazomib was not covered by health insurance, participants faced increased financial burdens. Moreover, a wide range of the 95% CI for the primary endpoint suggests uncertainty

in the precision of the estimates, affecting the robustness of the study results. The PFS observed in our study was shorter than expected with BR or continuous zanubrutinib. Another limitation is the toxicity burden related to ixazomib and dexamethasone, which could have adversely affected patient outcomes and adherence. Lastly, the patients' deep remission rate is high but they cannot discontinue the drug safely with high IgM rebound could be a reason for treatment termination. These factors underscore the need for further investigation and caution in drawing definitive conclusions from this study.

In conclusion, this study showed that ZID might be a good choice for patients who want to receive time-limited BTKi therapy with high deep response and good safety.

Acknowledgments

This study was supported by grants from the National Nature Science Foundation of China (82370197, WJ Xiong; 82170193 and 82200215, YT Yan) and the Chinese Academy of Medical Sciences Innovation Fund for Medical Sciences (2022-I2M-1-022, LG Qiu).

The authors thank the patients, investigators, and research staff who participated in the study. Thanks to BeiGene .Ltd for providing zanubrutinib for this study. The authors thank Dr. Natasha Aggarwal and Dr. Priyanka Bannikoppa of Indegene Pvt Ltd, India, for their editorial assistance.

Authorship

WX, TY, TW, and WS contributed equally to this work. SY and LQ contributed equally to this work. SY and LQ designed the study; WX analyzed the data,

performed statistical analyses, and wrote the manuscript; YY, TW, and WS collected data. YY, TY, RL suggested revisions; YW,WL,HL,GA,YX ,WH and DZ acquired data, managed patients and suggested revisions; LQ and SY revised the manuscript critically and approved the final version.

References

1. Treon SP, Tedeschi A, San-Miguel J, et al. Report of consensus Panel 4 from the 11th International Workshop on Waldenstrom's macroglobulinemia on diagnostic and response criteria. *Semin Hematol* 2023; **60**(2): 97-106.

2. Treon SP, Sarosiek S, Castillo JJ. How I use genomics and BTK inhibitors in the treatment of Waldenström macroglobulinemia. *Blood* 2024; **143**(17): 1702-12.

3. Moreno DF, de Larrea CF, Castillo JJ. New treatment strategies for Waldenström macroglobulinemia. *Clin Adv Hematol Oncol* 2022; **20**(8): 506-15.

4. Treon SP, Xu L, Guerrera ML, et al. Genomic Landscape of Waldenström Macroglobulinemia and Its Impact on Treatment Strategies. *J Clin Oncol* 2020; **38**(11): 1198-208.

5. Buske C, Castillo JJ, Abeykoon JP, et al. Report of consensus panel 1 from the 11(th) International Workshop on Waldenstrom's Macroglobulinemia on management of symptomatic, treatment-naïve patients. *Semin Hematol* 2023; **60**(2): 73-9.

6. Laribi K, Poulain S, Willems L, et al. Long-term results of Waldenström macroglobulinaemia treatment by bendamustine and rituximab: A study on behalf of the French Innovative Leukemia Organization (FILO). *Br J Haematol* 2024; **204**(6): 2233-6.

7. Kumar SK, Callander NS, Adekola K, et al. Waldenström Macroglobulinemia/Lymphoplasmacytic Lymphoma, Version 2.2024, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw* 2024; **22**(1d): e240001.

8. Gavriatopoulou M, García-Sanz R, Kastritis E, Morel P, Dimopoulos MA. BDR in newly diagnosed patients with WM: Final analysis of a phase 2 study after a minimum follow-up of 6 years. *Blood* 2016; **129**(4): 456.

9. Treon SP, Meid K, Gustine J, et al. Long-Term Follow-Up of Ibrutinib Monotherapy in Symptomatic, Previously Treated Patients With Waldenström Macroglobulinemia. *J Clin Oncol* 2021; **39**(6): 565-75.

10. Treon SP, Tripsas CK, Meid K, et al. Ibrutinib in previously treated Waldenström's macroglobulinemia. *N Engl J Med* 2015; **372**(15): 1430-40.

11. Castillo JJ, Meid K, Gustine JN, et al. Long-term follow-up of ibrutinib monotherapy in treatment-naive patients with Waldenstrom macroglobulinemia. *Leukemia* 2022; **36**(2): 532-9.

12. Dimopoulos MA, Opat S, D'Sa S, et al. Zanubrutinib Versus Ibrutinib in

549 Symptomatic Waldenström Macroglobulinemia: Final Analysis From the
550 Randomized Phase III ASPEN Study. *J Clin Oncol* 2023; **41**(33): 5099-106.
551 13. Buske C, Jurczak W, Salem JE, Dimopoulos MA. Managing
552 Waldenström's macroglobulinemia with BTK inhibitors. *Leukemia* 2023; **37**(1):
553 35-46.
554 14. Castillo JJ, Xu L, Gustine JN, et al. CXCR4 mutation subtypes impact
555 response and survival outcomes in patients with Waldenström
556 macroglobulinaemia treated with ibrutinib. *Br J Haematol* 2019; **187**(3):
557 356-63.
558 15. Gustine JN, Meid K, Dubeau T, et al. Ibrutinib discontinuation in
559 Waldenström macroglobulinemia: Etiologies, outcomes, and IgM rebound. *Am*
560 *J Hematol* 2018; **93**(4): 511-7.
561 16. Abeykoon JP, Zanwar S, Ansell SM, et al. Ibrutinib monotherapy outside of
562 clinical trial setting in Waldenström macroglobulinaemia: practice patterns,
563 toxicities and outcomes. *Br J Haematol* 2020; **188**(3): 394-403.
564 17. Castillo JJ, Meid K, Flynn CA, et al. Ixazomib, dexamethasone, and
565 rituximab in treatment-naive patients with Waldenström macroglobulinemia:
566 long-term follow-up. *Blood Adv* 2020; **4**(16): 3952-9.
567 18. Dasmahapatra G, Patel H, Dent P, Fisher RI, Friedberg J, Grant S. The
568 Bruton tyrosine kinase (BTK) inhibitor PCI-32765 synergistically increases

proteasome inhibitor activity in diffuse large-B cell lymphoma (DLBCL) and mantle cell lymphoma (MCL) cells sensitive or resistant to bortezomib. *Br J Haematol* 2013; **161**(1): 43-56.

19. Castillo JJ, Buske C, Trotman J, Sarosiek S, Treon SP. Bruton tyrosine kinase inhibitors in the management of Waldenström macroglobulinemia. *Am J Hematol* 2023; **98**(2): 338-47.

20. Xiong W, Wang Z, Wang T, et al. Minimal residual disease status improved the response evaluation in patients with Waldenström's macroglobulinemia. *Front Immunol* 2023; **14**: 1171539.

21. Yan Y, Yu Y, Xiong W, et al. Determination of MYD88 and CXCR4 Mutations for Clinical Detection and Their Significance in Waldenström Macroglobulinemia. *Clin Cancer Res* 2024; **30**(23): 5483-93.

22. Owen RG, Kyle RA, Stone MJ, et al. Response assessment in Waldenström macroglobulinaemia: update from the VIth International Workshop. *Br J Haematol* 2013; **160**(2): 171-6.

23. Parrondo RD, Dutta N, LaPlant BR, et al. A phase II study of ibrutinib in combination with ixazomib in patients with Waldenström macroglobulinaemia. *Br J Haematol* 2024; **204**(5): 1825-9.

24. Dimopoulos MA, Tedeschi A, Trotman J, et al. Phase 3 Trial of Ibrutinib plus Rituximab in Waldenström's Macroglobulinemia. *N Engl J Med* 2018;

378(25): 2399-410.

25. Flinn IW, van der Jagt R, Kahl B, et al. First-Line Treatment of Patients With Indolent Non-Hodgkin Lymphoma or Mantle-Cell Lymphoma With Bendamustine Plus Rituximab Versus R-CHOP or R-CVP: Results of the BRIGHT 5-Year Follow-Up Study. *J Clin Oncol* 2019; **37**(12): 984-91.

26. Rummel MJ, Niederle N, Maschmeyer G, et al. Bendamustine plus rituximab versus CHOP plus rituximab as first-line treatment for patients with indolent and mantle-cell lymphomas: an open-label, multicentre, randomised, phase 3 non-inferiority trial. *Lancet* 2013; **381**(9873): 1203-10.

27. Cholujova D, Beke G, Hunter ZR, et al. Dysfunctions of innate and adaptive immune tumor microenvironment in Waldenström macroglobulinemia. *Int J Cancer* 2023; **152**(9): 1947-63.

28. Kondo K, Shaim H, Thompson PA, et al. Ibrutinib modulates the immunosuppressive CLL microenvironment through STAT3-mediated suppression of regulatory B-cell function and inhibition of the PD-1/PD-L1 pathway. *Leukemia* 2018; **32**(4): 960-70.

Figure 1. The response rate of patients. The response rate of the overall patients and the patients with or without CXCR4 mutation

Figure 2. The changes in IgM, hemoglobin and bone marrow tumor load in patients before and after treatment

A-B. (A) The change of IgM and (B) hemoglobin before and after treatment. (C) The change of SPD before and after treatment. D-E. The malignant cells detected by (D) FCM and by (E) bone marrow biopsy. (F) The percentage of tumor cells of baseline, after induction and at best response. Hb: Hemoglobin; SPD：Maximum longest diameter of the tumor multiplied by the longest diameter perpendicular to it. FCM：flow cytometry.

Figure 3. The relationship between Immune microenvironment and the lymphocyte subsets

A-C. The relationship between the level of IgM and the percentage of (A) CD3, (B) CD4 and (C)NK cells. (D) The percentage of CD4 in patients who achieved VGPR or not. E-F. The percentage of (E) CD4 and (F) CD8 before and after treatment.

Figure 4. Kaplan-Meier curves of survival and response analyses

(A) The Kaplan-Meier curves of PFS as determined by investigator assessment. Shaded area indicates 95% CI. (B) The Kaplan-Meier curves of OS. Shaded area indicates 95% CI. (C) PFS in patients with CXCR4 mutation or CXCR4 wildtype. (D) OS in patients with CXCR4 mutation or CXCR4 wildtype.

Table 1. The Clinical characteristics of enrolled WM patients

Characteristic	N = 24
Age	
Median (range), years	62 (32-73)
≥65 years, n (%)	9 (37.5)
Gender	
Male, n (%)	17 (70.8)
Lymphadenopathy ≥1.5 cm, n (%)	7 (29.2)
Splenomegaly, n (%)	10 (41.7)
Serum IgM level	
Median (range), g/L	41.9 (6.4-125.0)
>40 g/L, n (%)	13 (54.2)
MYD88^{L265P} mutation, n (%)	23 (95.8)
CXCR4 mutation, n (%)	5 (21.7)
Hemoglobin level	
Median (range), g/L	87.0 (68.0-127.0)
≤110, n (%)	19 (79.2)
Platelet count	
Median (range), 10^9/L	164 (27-435)
≤100, n (%)	5 (20.8)
Serum β2-microglobulin	
Median (range), mg/L	3.5 (2.0-15.8)
>3 mg/L, n (%)	15 (62.5)
Serum albumin	
Median (range), g/L	35.8 (23.4-45.4)
＜35 g/L, n (%)	9 (37.5)
With B symptom	5 (20.8)
ISSWM score	
Low risk, n (%)	4 (16.7)
Intermediate risk, n (%)	14 (58.3)
High risk, n (%)	6 (25.0)

CXCR4, CXC-chemokine receptor 4; ISSWM, International Prognostic Scoring System for WM; MYD88, myeloid differentiation primary response gene 88.

Table 2. The adverse events of patients

Adverse events	Any grade	Grade 1	Grade 2	Grade 3	Grade 4
Blood and lymphatic system disorders					
Neutropenia	8 (33.3)	4 (16.7)	4 (16.7)	0	0
Thrombocytopenia	7 (29.2)	2 (8.3)	3 (12.5)	2 (8.3)	0
Leukopenia	4 (16.7)	3 (12.5)	1 (4.2)	0	0
Respiratory, thoracic, and mediastinal disorders					
Epistaxis	3 (12.5)	2 (8.3)	1 (4.2)	0	0
Cough	3 (12.5)	2 (8.3)	1 (4.2)	0	0
Dyspnea	2 (8.3)	1 (4.2)	1 (4.2)	0	0
Gingival bleeding	1 (4.2)	1 (4.2)	0	0	0
Gastrointestinal disorders					
Stomach ache	2 (8.3)	2 (8.3)	0	0	0
Diarrhea	2 (8.3)	2 (8.3)	0	0	0
Elevated aminotransferase	1 (4.2)	1 (4.2)	0	0	0
Vomiting	1 (4.2)	1 (4.2)	0	0	0
Nausea	1 (4.2)	1 (4.2)	0	0	0
Anorexia	1 (4.2)	1 (4.2)	0	0	0
Dyspepsia	1 (4.2)	1 (4.2)	0	0	0
Vascular disorders					
Hypertension	2 (8.3)	1 (4.2)	1 (4.2)	0	0
Arrhythmia	1 (4.2)	1 (4.2)	0	0	0
Infections and infestations					
Fever	5 (20.8)	2 (8.3)	3 (12.5)	0	0
Infection	4 (16.7)	1 (4.2)	0	1 (4.2)	2 (8.3)
Herpes zoster	2 (8.3)	1 (4.2)	1 (4.2)	0	0
Skin and subcutaneous tissue disorders					
Rash	4 (16.7)	2 (8.3)	2 (8.3)	0	0

Pruritus	3 (12.5)	2 (8.3)	0	1 (4.2)	0
Bleeder	3 (12.5)	3 (12.5)	0	0	0
Nervous system disorders					
Insomnia	5 (20.8)	1 (4.2)	3 (12.5)	1 (4.2)	0
Fatigue	4 (16.7)	2 (8.3)	2 (8.3)	0	0
Peripheral neuropathy	4 (16.7)	3 (12.5)	1 (4.2)	0	0
Paresthesia	3 (12.5)	3 (12.5)	0	0	0
Blur	2 (8.3)	2 (8.3)	0	0	0
Dizziness	2 (8.3)	2 (8.3)	0	0	0
Headaches	1 (4.2)	1 (4.2)	0	0	0
Metabolism					
Hyperglycemia	3 (12.5)	1 (4.2)	1 (4.2)	1 (4.2)	0
Edema	2 (8.3)	1 (4.2)	1 (4.2)	0	0
Osteoporosis	1 (4.2)	1 (4.2)	0	0	0

Figure 1

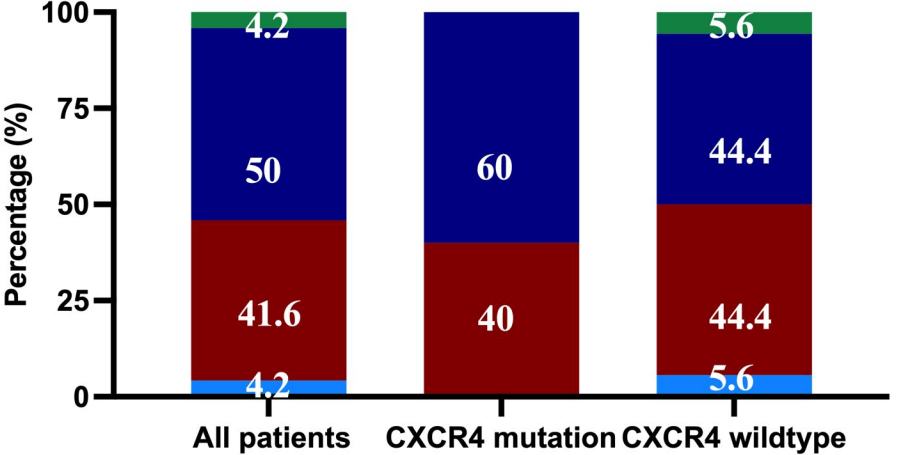

Figure 2

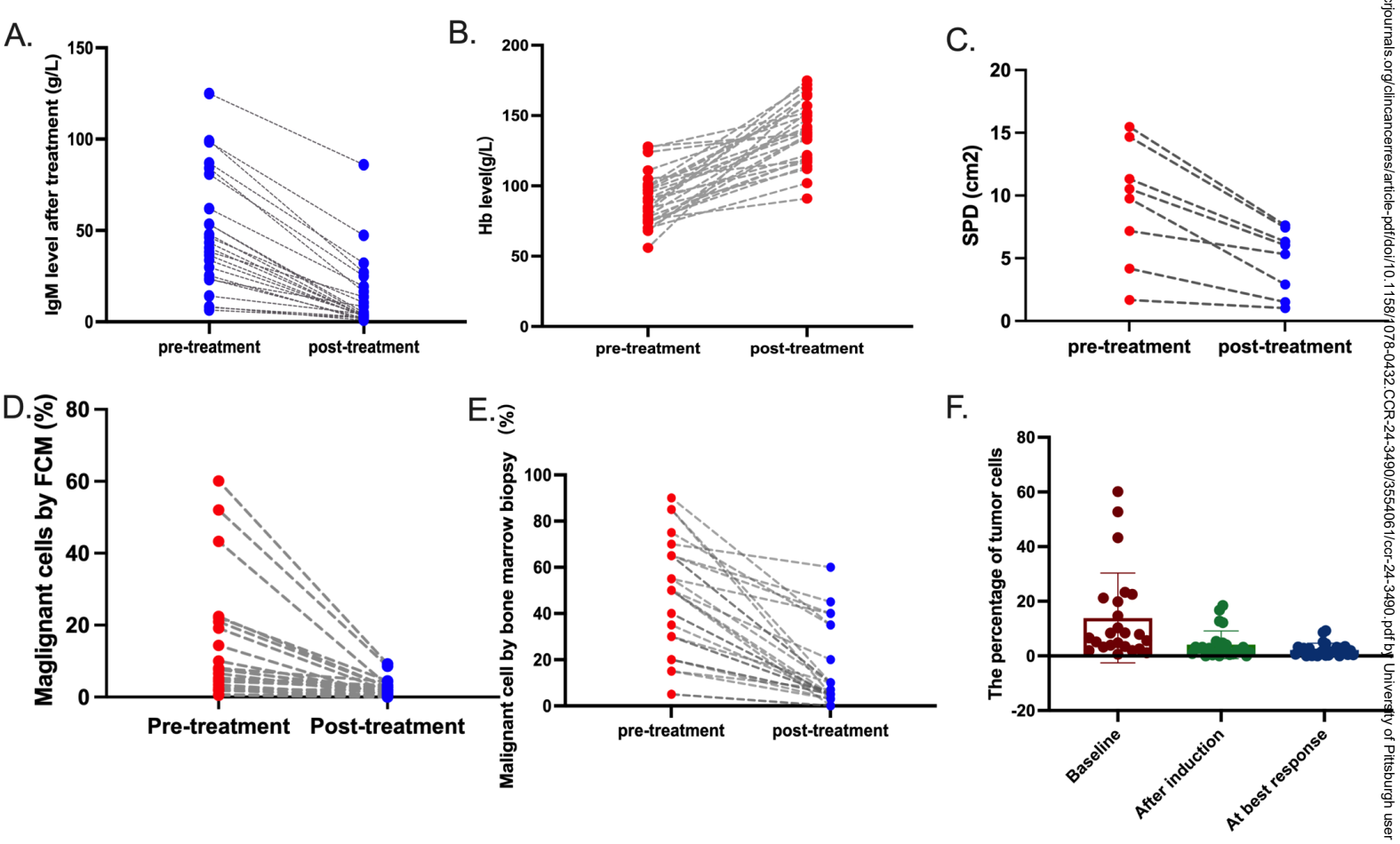

Figure 3

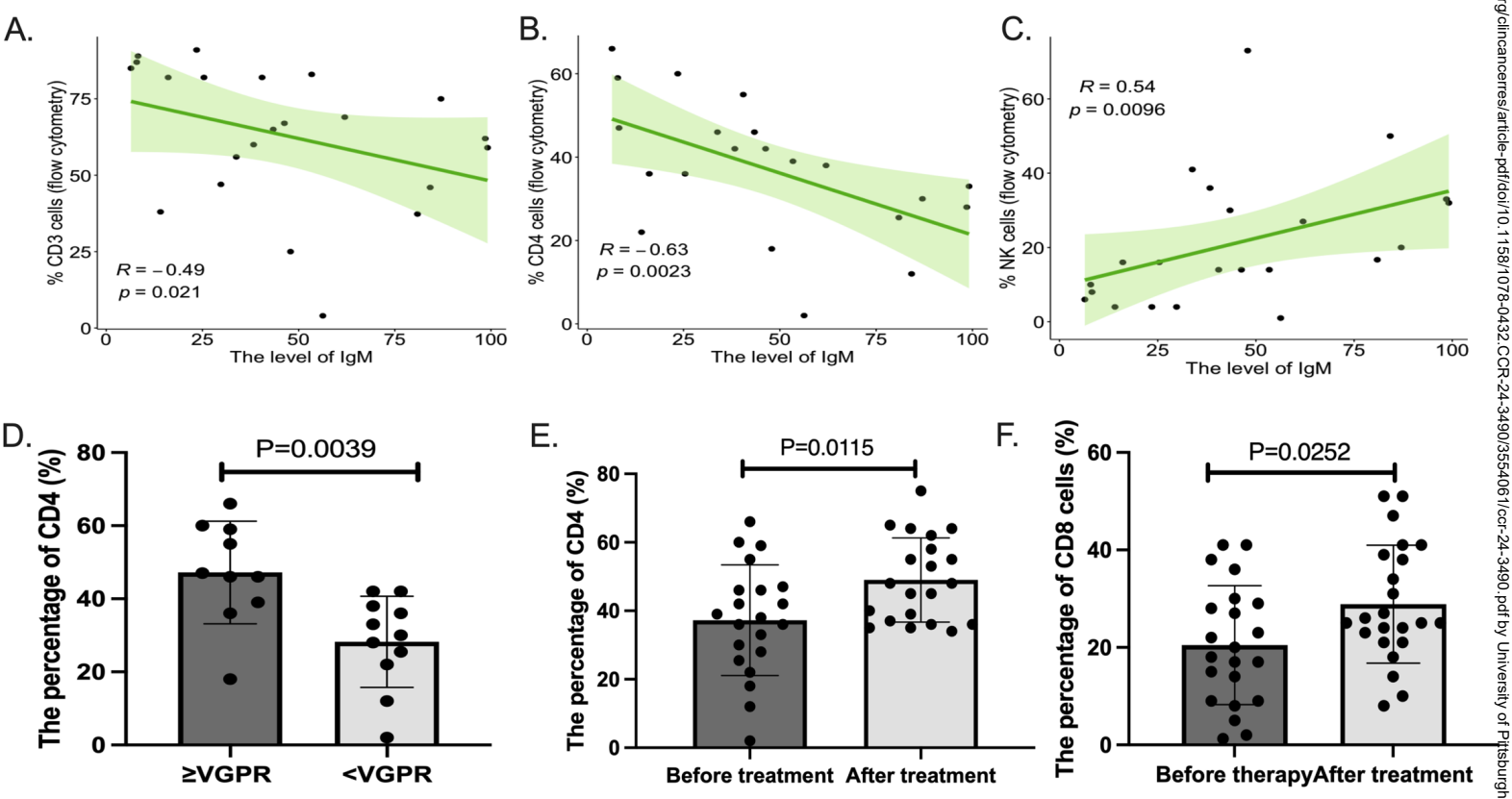

Figure 4