ORIGINAL ARTICLE

Ibrutinib plus Venetoclax for the Treatment of Mantle-Cell Lymphoma

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ABSTRACT

BACKGROUND

Both the BTK inhibitor ibrutinib and the BCL2 inhibitor venetoclax are active as monotherapy in the treatment of mantle-cell lymphoma. Complete response rates of 21% have been observed for each agent when administered as long-term continuous therapy. Preclinical models predict synergy in combination.

METHODS

We conducted a single-group, phase 2 study of daily oral ibrutinib and venetoclax in patients, as compared with historical controls. Patients commenced ibrutinib monotherapy at a dose of 560 mg per day. After 4 weeks, venetoclax was added in stepwise, weekly increasing doses to 400 mg per day. Both drugs were continued until progression or an unacceptable level of adverse events. The primary end point was the rate of complete response at week 16. Minimal residual disease (MRD) was assessed by flow cytometry in bone marrow and by allele-specific oligonucleotide–polymerase chain reaction (ASO-PCR) in blood.

RESULTS

The study included 24 patients with relapsed or refractory mantle-cell lymphoma (23 patients) or previously untreated mantle-cell lymphoma (1 patient). Patients were 47 to 81 years of age, and the number of previous treatments ranged from none to six. Half the patients had aberrations of *TP53*, and 75% had a high-risk prognostic score. The complete response rate according to computed tomography at week 16 was 42%, which was higher than the historical result of 9% at this time point with ibrutinib monotherapy ($P<0.001$). The rate of complete response as assessed by positron-emission tomography was 62% at week 16 and 71% overall. MRD clearance was confirmed by flow cytometry in 67% of the patients and by ASO-PCR in 38%. In a time-to-event analysis, 78% of the patients with a response were estimated to have an ongoing response at 15 months. The tumor lysis syndrome occurred in 2 patients. Common side effects were generally low grade and included diarrhea (in 83% of the patients), fatigue (in 75%), and nausea or vomiting (in 71%).

CONCLUSIONS

In this study involving historical controls, dual targeting of BTK and BCL2 with ibrutinib and venetoclax was consistent with improved outcomes in patients with mantle-cell lymphoma who had been predicted to have poor outcomes with current therapy. (Funded by Janssen and others; AIM ClinicalTrials.gov number, NCT02471391.)

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Mantle-cell lymphoma is a B-cell cancer with a median survival of 3 to 6 years.[1-3] Younger patients without coexisting conditions are treated with intensive chemotherapy,[4] which is commonly consolidated by autologous stem-cell transplantation,[5,6] whereas older patients or those with coexisting conditions are treated with chemotherapy and maintenance rituximab.[7] Until recently, patients with chemotherapy-resistant disease had few effective treatment options.[8-10]

Recent treatment advances have included the emergence of targeted therapies for B-cell neoplasms. Ibrutinib and venetoclax (ABT-199) are two of the most active agents in the treatment of chronic lymphocytic leukemia and mantle-cell lymphoma.[11-13] Ibrutinib is an irreversible inhibitor of Bruton's tyrosine kinase (BTK), an integral component of the B-cell–receptor pathway, which is often co-opted by B-cell cancers with resultant excessive growth signaling.[14,15] A phase 2 study of ibrutinib involving patients with relapsed or refractory mantle-cell lymphoma showed a best overall response rate of 68%, a rate of complete response of 21%, and a median progression-free survival of 13.9 months, and a phase 3 trial showed a best overall response rate of 72%, a rate of complete response of 19%, and a median progression-free survival of 14.6 months.[11,12] In the phase 3 trial, ibrutinib was superior to temsirolimus with regard to response rates, safety profile, and progression-free survival.[12] Common or serious side effects of ibrutinib include bleeding due to platelet dysfunction,[16] diarrhea, rash, and atrial fibrillation.[11,12] Resistance to ibrutinib in mantle-cell lymphoma is often related to activating mutations in the nuclear factor κB (NF-κB) pathway,[17,18] whereas in resistant chronic lymphocytic leukemia, mutations of *BTK* and *PLCγ2*[19] are observed in association with resistance.

Venetoclax is a BH3-mimetic agent that directly and specifically inhibits BCL2,[20,21] inducing apoptosis in malignant cells when BCL2 is overexpressed.[23] In a phase 1 study, venetoclax had greatest single-agent activity, among B-cell cancers, against chronic lymphocytic leukemia[21] and mantle-cell lymphoma,[13] both of which highly express BCL2. Across a range of doses in patients with relapsed or refractory mantle-cell lymphoma, a best overall response rate of 75% and a complete response rate of 21% were reported, with a median progression-free survival of 14 months.[13] An important side effect of venetoclax in early trials was the occurrence of the tumor lysis syndrome in patients with a large disease burden.[13,21] Other side effects included gastrointestinal symptoms and neutropenia.[13,21] The mechanisms for venetoclax resistance in patients are not well defined.

Preclinical models indicate that dual inhibition of BTK and BCL2 is synergistic.[24-26] Ibrutinib and venetoclax affect different critical pathways in the malignant B cell and have overlapping toxic effects that are generally minor, thus allowing for the development of an oral combination therapy with the potential for improved efficacy. In a historically controlled study, we investigated this combination therapy in patients with mantle-cell lymphoma.

METHODS

STUDY DESIGN AND PATIENT POPULATION

The ABT-199 and Ibrutinib in Mantle-Cell Lymphoma (AIM) study was an investigator-initiated, open-label, single-group, phase 2 study. Patients were enrolled at two sites in Melbourne, Australia. Adult patients were eligible if they had relapsed or refractory mantle-cell lymphoma or, if they had previously untreated mantle-cell lymphoma, were not suitable candidates for cytotoxic chemotherapy. Patients also had to have a neutrophil count of at least 750 per cubic millimeter, a platelet count of at least 50,000 per cubic millimeter, a creatinine clearance of at least 50 ml per minute, and an Eastern Cooperative Oncology Group performance-status score of 0, 1, or 2. (The ECOG performance-status score is assessed on a 5-point scale, with higher numbers indicating greater disability. A score of 0 indicates that the patient is fully active, a score of 1 that the patient is ambulatory but is restricted in physically strenuous activity, and a score of 2 that the patient is ambulatory, capable of self-care, and confined to a bed or chair for <50% of waking hours.) The key eligibility criteria are listed in the Supplementary Appendix, available with the full text of this article at NEJM.org.

TREATMENT

To reduce the risk of the tumor lysis syndrome, all patients commenced treatment with ibrutinib monotherapy at an oral dose of 560 mg per day for the first 4 weeks. Venetoclax was then intro-

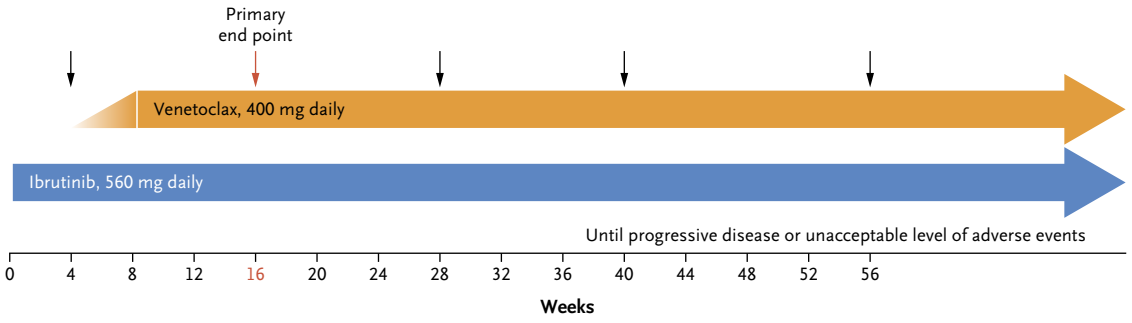

Figure 1. Study Schema.

Patients received ibrutinib at a dose of 560 mg per day for 4 weeks, before the weekly dose escalation of venetoclax to a target dose of 400 mg per day began. Both drugs were continued until disease progression or an unacceptable level of adverse events occurred. Disease reassessment was performed at weeks 4, 16, 28, 40, and 56 (arrows) by means of CT, with positron-emission tomography (PET) at baseline and at weeks 16 and 56, by means of bone marrow examination, and by means of allele-specific oligonucleotide–polymerase chain reaction (ASO-PCR) assessment of minimal residual disease in peripheral blood.

duced in week 5 according to a dosing schedule that started at 50 mg per day orally and increased weekly in a stepwise fashion (ramp-up) to 100 mg per day, then to 200 mg per day, and finally to 400 mg per day, on the basis of the recommended dose for treating chronic lymphocytic leukemia at the time of study conception (Fig. 1). Subsequently, the recommended phase 2 dose in mantle-cell lymphoma was reported to be 800 mg per day,[13] and the study protocol (available at NEJM.org) was amended to allow escalation to a dose of 800 mg per day after week 16 if a complete response had not occurred.

Risk stratification for the tumor lysis syndrome was adapted from that used for studies of venetoclax in patients with chronic lymphocytic leukemia.[21,22] Patients at high risk were those who had a largest tumor dimension of 10 cm or more or who had a combination of the largest tumor dimension of 5 cm or more and a circulating lymphocyte count of at least 25,000 per cubic millimeter. Patients at medium risk were those who either had a largest tumor dimension of 5 cm or more or had a circulating lymphocyte count of at least 25,000 per cubic millimeter. Patients at low risk had neither clinical characteristic. After the occurrence of the tumor lysis syndrome in 2 of the first 15 patients, the protocol was amended to include an additional week of venetoclax ramp-up, starting at 20 mg per day. Key elements of monitoring for the tumor lysis syndrome and study amendments are provided in the Supplementary Appendix. After the completion of the venetoclax ramp-up period, all the patients continued both ibrutinib and venetoclax daily until disease progression or until an unacceptable level of adverse events occurred.

ASSESSMENTS

Disease reassessment was performed at weeks 4, 16, 28, 40, and 56 by means of computed tomography (CT), with and without the use of ^{18}F-fluorodeoxyglucose–positron-emission tomography (PET) (with disease graded with the use of the Deauville scale, on which scores range from 1 to 5, with lower scores indicating less glucose metabolism; a score of 1 to 3 indicated a complete metabolic response[27,28]), by means of bone marrow examination (until minimal residual disease in bone marrow was found to be less than 1 in 10^4 cells), and by means of allele-specific oligonucleotide–polymerase chain reaction (ASO-PCR) assessment of minimal residual disease in peripheral blood. The response criteria for the imaging assessments are provided in the Supplementary Appendix. Patients with known gastrointestinal involvement underwent gastroscopy and colonoscopy, with random biopsies, to confirm complete response. Testing for minimal residual disease was performed by eight-color flow cytometry in the bone marrow,[29] with a minimum sensitivity of 10^{-3} to 10^{-4}, and by ASO-PCR assays in blood and bone marrow targeting either the clonal IgH rearrangement or t(11;14) translocation, designed to reach a sensitivity of 10^{-5}.[30,31]

Responses were centrally reviewed by the prin-

cipal investigator and a PET expert. Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.[32] The Mantle Cell Lymphoma International Prognostic Index (MIPI), which is used to assess risk on the basis of age, ECOG performance-status score, lactate dehydrogenase level, and white-cell count, was calculated with the use of published indexes.[33] Targeted amplicon sequencing of baseline tumor DNA (from pretreatment bone marrow or nodal biopsy specimens) and matched germline DNA was performed across 41 genes that are known to be recurrently mutated in patients with lymphoma (see the Supplementary Appendix).

STUDY OVERSIGHT

The study protocol was designed by the first author and the last two authors; the Peter MacCallum Cancer Centre had overall responsibility for the study and its conduct. Janssen and AbbVie approved the study design, provided study drugs and partial funding, and had an opportunity to review the manuscript, but they had no role in the conduct of the study or the collection and analysis of data and had no influence on the content of the manuscript or the decision to submit the manuscript for publication. The Human Research Ethics Committee at the Peter MacCallum Cancer Centre approved the protocol and all the amendments. The study was conducted according to the Declaration of Helsinki and the Good Clinical Practice guidelines of the International Conference on Harmonisation. All the patients provided written informed consent. The authors vouch for the adherence of the study to the protocol and for the accuracy and completeness of the data.

STATISTICAL ANALYSIS

The primary end point was the rate of complete response at week 16, as determined with the use of all the tests other than PET, in order to allow for comparison with the results of the PCYC-1104-CA phase 2 study of single-agent ibrutinib in mantle-cell lymphoma.[11] The PCYC-1104-CA study did not use PET for restaging, and the rate of complete response at week 16 was 9%.[34] Our study was designed to have 80% power to reject a complete response rate of 9% (at a one-sided alpha level of 0.05) if the rate of complete response was at least 30%. The data reported herein are as of October 4, 2017, and patients who did not have an event by this date had their data censored at the time of the last clinical assessment for time-to-event end points. Kaplan–Meier methods were used for time-to-event analyses. Response rates were described with exact 95% confidence intervals that were based on binomial distribution.

RESULTS

CHARACTERISTICS AND DISPOSITION OF THE PATIENTS

From July 2015 through September 2016, a total of 24 consecutive eligible patients were enrolled in the study. Among these patients, 23 had relapsed or refractory mantle-cell lymphoma, with a median of two previous therapies (range, one to six), and 1 patient had previously untreated mantle-cell lymphoma and could not undergo cytotoxic chemotherapy because the patient declined blood transfusions and there was both *TP53* mutation and deletion in the lymphoma (Table 1). The median age of the patients was 68 years (range, 47 to 81). Half the patients (including the previously untreated patient) had a *TP53* aberration, and 25% had an NF-κB pathway mutation (Table 1).

The disposition of the patients is listed in Table S1 in the Supplementary Appendix. The median time that the patients received study treatment was 14.4 months (range, 0.7 to 25.0), and the median follow-up was 15.9 months (range, 1.4 to 26.2). Two patients never received venetoclax owing to rapid disease progression during ibrutinib monotherapy in one patient and fatal infection in the other. All the patients without progression completed assessments, including PET scans, at week 56. As of October 4, 2017, a total of 16 patients remained in the study, including 2 patients with disease progression who continued study treatment after progression with ongoing clinical benefit.[35] A total of 8 patients discontinued treatment owing to disease progression (6 patients) or death without disease progression (2 patients).

EFFICACY

The responses according to an intention-to-treat analysis, with and without PET, are shown in Table 2. At week 16 (primary end point), the rate of complete response as assessed without PET was 42% (95% confidence interval [CI], 22 to 63), as compared with a complete response rate of 9% at the same time recorded in a historical cohort of patients who received ibrutinib monotherapy ($P<0.001$). Including PET assessments, the overall

Table 1. Characteristics of All 24 Patients at Study Entry.*

Characteristic	Value
Median age (range) — yr	68 (47–81)
Sex — no. (%)	
Female	3 (12)
Male	21 (88)
Previous treatment for mantle-cell lymphoma — no. (%)	
Yes	23 (96)
No†	1 (4)
No. of previous therapies among patients who had received therapy — median (range)‡	2 (1–6)
Disease refractory to most recent therapy — no./total no. (%)	11/23 (48)
Previous therapy — no./total no. (%)‡	
Autologous transplantation	7/23 (30)
Rituximab	23/23 (100)
Anthracycline	21/23 (91)
High-dose cytarabine	11/23 (48)
Bendamustine	4/23 (17)
ECOG performance-status score — no. (%)	
0	9 (38)
1	10 (42)
2	5 (21)
Bone marrow involvement by mantle-cell lymphoma — no. (%)	
Bone marrow involved at study entry	13 (54)
Negative at study entry but positive at wk 4	7 (29)
No bone marrow involvement	4 (17)
Bulky adenopathy — no. (%)	
≥5 cm to <10 cm	4 (17)
≥10 cm	2 (8)
Mantle Cell Lymphoma International Prognostic Index score category — no. (%)	
Low risk	1 (4)
Intermediate risk	5 (21)
High risk	18 (75)
Blastic or pleomorphic mantle-cell lymphoma — no./total no. (%)	1/21 (5)
Ki-67 ≥30% — no./total no. (%)	9/21 (43)
TP53 status — no. (%)	
Mutated with deletion	4 (17)
Mutated without deletion	7 (29)
Deletion without mutation	1 (4)
NF-κB pathway mutations in *CARD11*, *BIRC3*, or *TRAF2* — no. (%)	6 (25)
Tumor lysis risk category — no. (%)§	
Low risk	11 (46)
Intermediate risk	6 (25)
High risk	7 (29)

* Percentages may not total 100 because of rounding. ECOG denotes Eastern Cooperative Oncology Group.
† The patient did not receive cytotoxic chemotherapy because the patient declined blood transfusions and had a *TP53* mutation with deletion in mantle-cell lymphoma.
‡ Elective high-dose chemotherapy and autologous stem-cell transplantation after induction therapy were counted as a single first-line treatment. Full details of the previous therapies in the individual patients are provided in Table S1 in the Supplementary Appendix.
§ The two primary risk factors for the tumor lysis syndrome were the following: largest tumor dimension of 5 cm or more, and a circulating lymphocyte count of at least 25,000 per cubic millimeter. Low risk was defined as having neither risk factor, intermediate risk as having one risk factor, and high risk as having both risk factors or as having a largest tumor dimension of 10 cm or more. Three patients in the high-risk subgroup had a reduction in the risk of the tumor lysis syndrome after 4 weeks of ibrutinib monotherapy.

response rate at week 16 was 71% (95% CI, 49 to 87), with a complete response occurring in 62% of the patients and a partial response in 8%. Two patients who had a partial response at week 16 subsequently had a complete response, one of which occurred after the escalation of the venetoclax dose to 800 mg per day. Overall, 17 patients (71%; 95% CI, 49 to 87) had a PET-confirmed complete response as their best response (Table 2).

The kinetics of the response are shown in Figure 2. Ibrutinib reduced the nodal bulk by 25% or more at week 4 in 14 of 21 patients who could be assessed (Fig. 2A), and the risk category for the tumor lysis syndrome was downgraded in 3 of 7 patients who had been at high risk at study

Table 2. Responses to Combination Therapy with Ibrutinib and Venetoclax.*

Response	Without PET (N=24)	With PET (N=24)
Overall		
Response at wk 4 — no. (%)		
Complete response	0	—
Unconfirmed complete response	1 (4)	—
Partial response	10 (42)	—
Stable disease	10 (42)	—
Progressive disease	2 (8)	—
Could not be evaluated	1 (4)†	—
Response at wk 16 — no. (%)		
Complete response	10 (42)	15 (62)
Unconfirmed complete response	4 (17)	—
Partial response	4 (17)	2 (8)
Stable disease	2 (8)	1 (4)
Progressive disease	3 (12)	4 (17)
Could not be evaluated	1 (4)‡	2 (8)‡§
Best response — no. (%)		
Complete response	16 (67)	17 (71)
Unconfirmed complete response	1 (4)	—
Partial response	1 (4)	0
Stable disease	4 (17)	2 (8)
Progressive disease	2 (8)	3 (12)
Could not be evaluated	0	2 (8)‡§
	With Flow Cytometry	**With ASO-PCR¶**
According to MRD clearance		
Tissue tested	Bone marrow	Blood
Never MRD positive — no./total no. tested	4/24	0/16‖
Could be potentially evaluated for MRD response — no.	19**	16
Response at wk 4 — no./total no. (%)		
MRD negative	0	0
MRD positive	19/19 (100)	13/13 (100)
Response at wk 16 — no./total no. (%)		
MRD negative	12/18 (67)	2/13 (15)††
MRD positive	6/18 (33)	11/13 (85)

Table 2. (Continued.)

Response	Without PET (N=24)	With PET (N=24)
Best response		
Among patients who could be evaluated for MRD response — no./total no. (%)		
MRD negative	16/19 (84)	9/16 (56)‡‡
MRD positive	3/19 (16)	7/16 (44)
In the total population — no. (%)		
MRD negative	16 (67)	9 (38)
MRD not negative§§	8 (33)	15 (62)

* Percentages may not total 100 because of rounding. ASO-PCR denotes allele-specific oligonucleotide–polymerase chain reaction, MRD minimal residual disease, and PET positron-emission tomography.
† This patient had disease that could be evaluated only by PET, bone marrow examination, and endoscopy of the gastrointestinal tract, and full disease reassessment did not occur until week 16 in this patient.
‡ One patient with stable disease died from infection at week 6 and so could not be evaluated for CT or PET response at the week 16 time point.
§ Disease was not able to be assessed by PET in one patient (for details, see the footnote to Table S1 in the Supplementary Appendix).
¶ ASO-PCR targeted either the clonal IgH rearrangement or t(11;14) translocation and was designed to reach a sensitivity of 10^{-5}, reported in accordance with EuroMRD guidelines.[30,31]
‖ Eight patients could not be evaluated by ASO-PCR because of uninformative molecular markers (in seven patients) or unavailability of suitable baseline sample (in one).
** One patient with bone marrow involvement at baseline had systemic progression before completing ibrutinib monotherapy and did not undergo a repeat biopsy.
†† Bone marrow was concurrently assessed by ASO-PCR in one patient, who was confirmed to have MRD-negative status.
‡‡ Bone marrow was concurrently assessed by ASO-PCR in six patients, all of whom were confirmed to have MRD-negative status.
§§ A status of "not negative" included patients who could not be evaluated for MRD clearance and those who remained MRD-positive.

entry. At the same time point, detectable disease increased in the bone marrow (Fig. 2B) and blood (Fig. 2C). Consistent with mobilization of tumor cells from nodal sites, this finding included newly detected involvement of bone marrow in 7 patients. Disease clearance in patients who had a response occurred after the introduction of venetoclax.

In the total population of 24 patients, absence of minimal residual disease in bone marrow, as assessed by flow cytometry, was recorded in 16 patients (67%) and in blood, as assessed by ASO-PCR, in 9 (38%) (Table 2). A total of 6 patients underwent ASO-PCR testing of blood and bone marrow, and all had negative results in both compartments. Not all the patients could be evaluated for minimal residual disease clearance, because of a lack of an informative molecular marker (in 7 patients) or the unavailability of a suitable baseline sample (in 1) or follow-up sample (in 1) or because they never had detectable minimal residual disease (in 4). Among the patients who could be evaluated for minimal residual disease clearance, 16 of 19 (84%) had clearance according to flow cytometry, and 9 of 16 (56%) had clearance according to ASO-PCR. Among patients with a complete response who could be evaluated for minimal residual disease, 14 of 15 (93%) had a negative status according to flow cytometry, and 9 of 11 (82%) had a negative status according to ASO-PCR.

After a median follow-up of 15.9 months, the median progression-free survival had not been reached, with estimated rates of progression-free survival of 75% (95% CI, 60 to 94) at 12 months and 57% (95% CI, 40 to 82) at 18 months (Fig. 3A). In total, eight patients had disease progression — five patients had disease that was primarily refractory to study therapy, and three had a relapse after having a complete response and while continuing therapy. Two of these instances of disease progression were clinically silent and were detected early owing to the stringent restaging procedures. One patient had low-volume nodal progression detected by PET at week 16, when

the findings on CT remained consistent with an unconfirmed complete response and the bone marrow was negative for minimal residual disease on flow cytometry. A second patient had a reappearance of lymphoid aggregates in bone marrow at week 56, when both the PET and CT scans showed an ongoing complete response. As of the time of analysis, the median duration of response had not been reached, and 78% (95% CI, 59 to 100) of the patients with a response were estimated to be progression-free at 15 months (Fig. 3B). The rate of overall survival was 79% (95% CI, 64 to 97) at 12 months and 74% (95% CI, 57 to 95) at 18 months (Fig. 3C).

SAFETY

The adverse events that occurred during treatment are summarized in Table 3. The most common toxic effects were gastrointestinal (diarrhea in 83% of the patients, nausea or vomiting in 71%, and gastroesophageal reflux in 38%). The worst grade of diarrhea was grade 1 in 38% of the patients, grade 2 in 33%, and grade 3 in 12%. Diarrhea was typically transient, with diarrhea of grade 2 or higher lasting a median of 2 weeks (range, <1 to 14), and it was managed with antimotility agents or with a dose reduction (of ibrutinib, venetoclax, or both) if it was persistent (Table S2 in the Supplementary Appendix). One patient with an ongoing complete response stopped ibrutinib because of diarrhea but continued venetoclax.

Other common toxic effects were fatigue (in 75% of the patients), bleeding, bruising, or postoperative hemorrhage (in 54%), musculoskeletal or connective-tissue pain (50%), cough or dyspnea (46%), soft-tissue infection (42%), upper respiratory tract infection (42%), neutropenia (33%), and lower respiratory tract infection (33%). With the exception of neutropenia, these events were predominantly of grade 1 or 2 in severity. Febrile neutropenia occurred in one patient. A total of seven patients (29%) received granulocyte colony-stimulating factor for neutropenia.

Serious adverse events were recorded in 14 patients (58%). The tumor lysis syndrome occurred in 2 of the first 15 patients who commenced venetoclax at a dose of 50 mg per day (both patients were in the high-risk subgroup) (Table S3 in the Supplementary Appendix). This situation prompted a protocol amendment to lower the starting dose of venetoclax to 20 mg daily. Subsequently, no cases of the tumor lysis syndrome were observed in 7 patients, including 2 who were in the high-risk subgroup, who commenced venetoclax at a dose of 20 mg per day.

The mean (±SD) relative dose intensities were 96±18% for venetoclax and 87±17% for ibrutinib. Overall, 15 patients had an adjustment to the ibrutinib or venetoclax dose (12 patients with ibrutinib and 7 with venetoclax, including 4 with both drugs) in the first 56 weeks (Table S2 in the Supplementary Appendix).

Six deaths occurred during the study, of which four were attributed to disease progression and two to other causes. One patient died from malignant otitis externa during week 6, after treatment with ibrutinib alone. The second patient died from cardiac failure while having an ongoing complete response. This patient had a history of anthracycline-related cardiomyopathy, and atrial fibrillation had developed at week 5. The patient received heart-rate–control medication and was stable according to regular monitoring, including echocardiography. Despite this, he presented during week 52 with severe heart failure, ceased all trial therapy, and died during week 55. Another patient who had anthracycline-related cardiomyopathy had symptomatic heart failure with a reduction in his left ventricular ejection fraction at week 20. Ibrutinib was stopped, and the patient continued to receive venetoclax monotherapy, with complete resolution of his symptoms and recovery of the left ventricular ejection fraction to the baseline level by week 40. On the basis of these patients, repeat echocardiography was performed in all the patients who had an abnormal left ventricular ejection fraction at baseline or who had symptoms that were suggestive of heart failure, and no further cases of heart failure were identified.

FEATURES ASSOCIATED WITH TREATMENT RESPONSE

To investigate factors associated with outcome, we categorized patients according to whether they had a response (18 patients who had a PET-confirmed complete response and 1 patient who could not be evaluated by PET but who was progression-free at 26 months) or did not have a response (5 patients, who had disease that was primarily refractory to study treatment). The patient who died from infection during week 6 was not included in this analysis. No associations were apparent between response and age, number of previous therapies, previous autologous transplan-

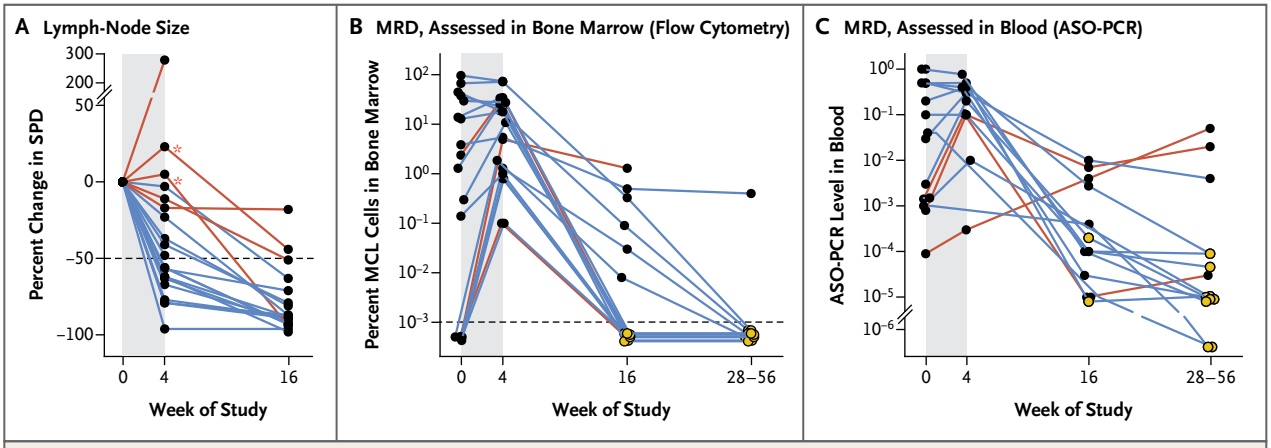

Figure 2. Kinetics of Response and Clearance of Minimal Residual Disease (MRD).

Panel A shows the patterns of change from baseline in lymph-node bulk as measured by the CT sum of perpendicular diameters (SPD) at weeks 4 and 16 relative to the baseline SPD. Blue lines indicate patients who had a partial or complete response at 16 weeks, and red lines indicate patients who had stable or progressive disease at 16 weeks. Red asterisks indicate the 2 patients who had the tumor lysis syndrome after the addition of venetoclax at a dose of 50 mg per day in week 5. The dashed line at –50 indicates a reduction in the nodal size commensurate with a partial response. The shaded area in all panels represents the period of ibrutinib monotherapy between study commencement and the end of week 4. Panels B and C show the absolute changes in the level of MRD in bone marrow (19 patients), as assessed by flow cytometry, and in peripheral blood (16 patients who had data that could be evaluated), as assessed by ASO-PCR. MCL denotes mantle-cell lymphoma. Samples from patients with MRD-negative status are indicated in yellow. Data from the 4 patients with MRD-negative bone marrow at entry and throughout the study and from the 1 patient who did not undergo restaging assessment of bone marrow are not shown in Panel B, and all MRD-negative samples of bone marrow are plotted below 10^{-3} (dashed line). For MRD in bone marrow, negative status refers to a value that was less than 10^{-4} in most samples, but when less than 400,000 cells were analyzed, the sensitivity was less than 10^{-3}. In Panel C, the threshold for MRD negativity in the blood as assessed by ASO-PCR varied according to the sample.

tation, ECOG performance-status score, bulky-node size, MIPI score, lactate dehydrogenase level, or *IGHV* mutational status (data not shown). Among the 5 patients who could be evaluated and had received previous treatment with three or more lines of therapy, 3 had a response. The 1 patient with documented pleomorphic mantle-cell lymphoma had disease that was refractory to study treatment. Patients with tumor expression of Ki-67 (a marker of cellular proliferation, as detected by immunohistochemical testing) of 30% or more (9 patients, including 5 with a response) were less likely to have a response than those with a lower Ki-67 level (12 patients, all of whom had a response; P=0.02).

Using targeted sequencing of prestudy tumor specimens, we identified mutations in all 24 patients (Table S4 in the Supplementary Appendix). Mutations in *ATM* were present in 10 patients, all of whom had a complete response. Responses were observed in patients who had mutations associated with ibrutinib resistance, including a complete response in 5 of 6 patients with NF-κB pathway mutations. Of the 12 patients who had a *TP53* mutation or deletion, 6 (50%) had a complete response, with 5 remaining progression-free for 13 to 20 months at the time of analysis. Details regarding *TP53* status and subsequent treatment are provided in Table S1 in the Supplementary Appendix.

DISCUSSION

The preclinical rationale for combining BTK inhibition with BCL2 inhibition is compelling for B-cell cancers in which substantial activity has been observed for each as monotherapy.[11-13] Ibrutinib and venetoclax can be administered in combination, with side effects that are acceptable to both patients and physicians, and the combination is highly active. A complete response was observed in 71% of the patients. Absence of minimal residual disease was documented in 67% of the patients according to flow cytometry and in 38% according to ASO-PCR (including 93% and 82%, respectively, of the patients who had a complete response and could be assessed). A total of 78% of the responses were ongoing at 15 months,

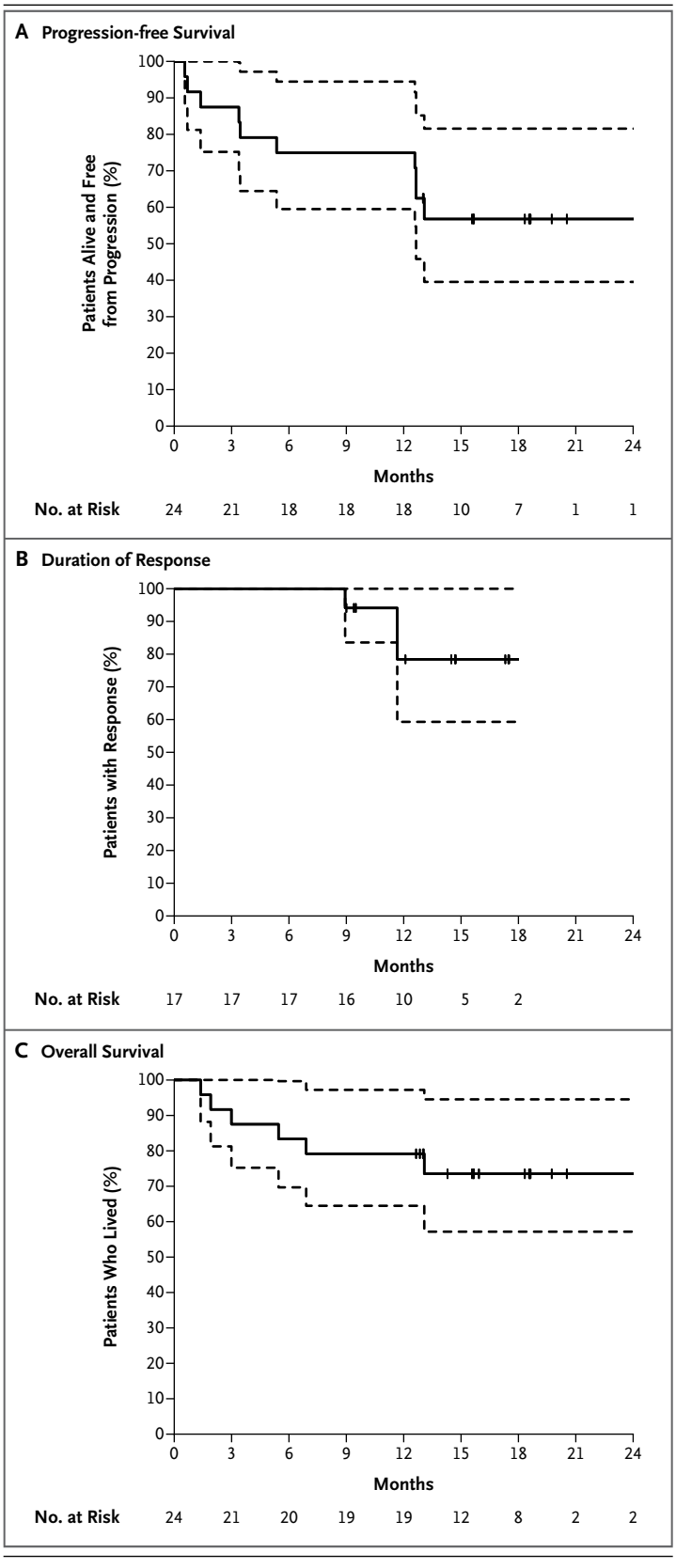

Figure 3. Key Survival Outcomes.

Panel A shows progression-free survival among all 24 patients. The rate of progression-free survival was estimated to be 75% (95% CI, 60 to 94) at 12 months and 57% (95% CI, 40 to 82) at 18 months. The dashed lines indicate the 95% confidence interval, and tick marks censored data. Panel B shows the duration of response among the 17 patients who had a response. The rate of ongoing response was estimated in the time-to-event analysis to be 78% (95% CI, 59 to 100) at 12 months and 78% (95% CI, 59 to 100) at 15 months. Panel C shows overall survival among all 24 patients. The overall survival rate was estimated to be 79% (95% CI, 64 to 97) at 12 months and 74% (95% CI, 57 to 95) at 18 months.

and 57% of the patients were alive and progression-free at 18 months. Such outcomes appear to be substantially better than those that have been reported for ibrutinib or venetoclax monotherapy.[11-13]

Responses to combination therapy with ibrutinib and venetoclax appeared to be independent of traditional risk markers such as a high level of lactate dehydrogenase or the MIPI score, including responses in 60% of the heavily pretreated patients. However, patients with highly proliferative disease (as indicated by a high Ki-67 level or by blastic or pleomorphic morphologic features) were less likely to have a complete response than patients without these characteristics. Resistance to ibrutinib and venetoclax may be overcome in preclinical experiments by the addition of other active agents such as proteasome inhibitors,[36] and such strategies may be tested in clinical trials. Preliminary reports of other combinations have also indicated high rates of complete response and minimal residual disease clearance,[37] and these may present therapeutic alternatives in patients with relapsed mantle-cell lymphoma.

TP53 mutations appear to be a critical prognostic factor in mantle-cell lymphoma, being strongly associated with treatment resistance and inferior survival among patients receiving intensive chemotherapy and undergoing first-line autologous stem-cell transplantation.[38,39] *TP53* aberrations were present in 50% of the patients in this study, half of whom had complete responses, most of which were durable. However, the number of such patients is small, and the follow-up is less than 2 years. Other studies have highlighted the role of mutations in the NF-κB pathway as mediators of a poor response to ibrutinib or chemo-

Table 3. Adverse Events and Serious Adverse Events.*

Event	Any Grade (N=24)	Grade 3 or Higher (N=24)
	no. of patients with event (%)	
Any adverse event	24 (100)	17 (71)
Diarrhea	20 (83)	3 (12)†
Fatigue	18 (75)	0
Nausea or vomiting	17 (71)	0
Bleeding, bruising, or postoperative hemorrhage	13 (54)	1 (4)
Musculoskeletal or connective-tissue pain	12 (50)	1 (4)
Cough or dyspnea	11 (46)	1 (4)
Soft-tissue infection	10 (42)	2 (8)‡
Upper respiratory tract infection	10 (42)	0
Gastroesophageal reflux	9 (38)	0
Neutropenia	8 (33)	8 (33)
Lower respiratory tract infection	8 (33)	2 (8)
Anemia	7 (29)	3 (12)
Rash	7 (29)	0
Oral mucositis	5 (21)	0
Cramps	5 (21)	0
Sensory peripheral neuropathy	5 (21)	0
Thrombocytopenia	5 (21)	4 (17)
Tumor lysis syndrome	2 (8)	2 (8)
Atrial fibrillation	2 (8)	2 (8)
Any serious adverse event§	14 (58)	—
Diarrhea	3 (12)¶	—
Tumor lysis syndrome	2 (8)	—
Atrial fibrillation	2 (8)	—
Pyrexia	2 (8)	—
Pleural effusion	2 (8)	—
Cardiac failure	1 (4)‡	—
Soft-tissue infection	1 (4)‡	—

* Listed are the adverse events that were reported in at least 15% of the patients, as well as events of special interest (the tumor lysis syndrome and atrial fibrillation).
† The three cases of grade 3 diarrhea lasted 4 days, 1 week, and 2 weeks.
‡ Data include one fatal adverse event. The two fatal events that were considered by the investigators to be unrelated to disease progression were soft-tissue infection (malignant otitis externa) and cardiac failure.
§ Listed are the serious adverse events that were reported in at least two patients, as well as fatal events.
¶ Data include one patient with microscopic colitis that had been diagnosed on the basis of colonoscopy and biopsy.

therapy.[17,18,40] Preexisting NF-κB pathway mutations appeared to have no effect on the response to combination therapy with ibrutinib and venetoclax in our study. The small number of patients in these genetically defined subgroups means that these conclusions are preliminary. Larger studies will be needed to determine their validity.

The results of our study, which used a historical cohort as a control, are consistent with the notion that the combination of ibrutinib and venetoclax is highly effective in mantle-cell lymphoma. The potential superiority of the combination therapy to single-agent ibrutinib in mantle-cell lymphoma is being formally tested in an

ongoing phase 3 study (ClinicalTrials.gov number, NCT03112174). The occurrence of complete responses with clearance of minimal residual disease raises the question of whether treatment may be stopped in selected patients — a strategy that has been effective in patients with chronic lymphocytic leukemia who received combination therapy with venetoclax and rituximab.[41] The question remains open, given that two patients with a minimal residual disease–negative complete response in our series had a relapse. We and others (EudraCT number, 2016-002293-12) are currently evaluating the feasibility of response-adapted treatment cessation.

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