MALT1 Inhibition Is Efficacious in Both Naïve and Ibrutinib-Resistant Chronic Lymphocytic Leukemia


Abstract

The clinical efficacy displayed by ibrutinib in chronic lymphocytic leukemia (CLL) has been challenged by the frequent emergence of resistant clones. The ibrutinib target, Bruton’s tyrosine kinase (BTK), is essential for B-cell receptor signaling, and most resistant cases carry mutations in BTK or PLCG2, a downstream effector target of BTK. Recent findings show that MI-2, a small molecule inhibitor of the para-caspase MALT1, is effective in preclinical models of another type of BCR pathway-dependent lymphoma. We therefore studied the activity of MI-2 against CLL and ibrutinib-resistant CLL. Treatment of CLL cells in vitro with MI-2 inhibited MALT1 proteolytic activity reduced BCR and NF-κB signaling, inhibited nuclear translocation of RelB and p50, and decreased Bcl-xL levels. MI-2 selectively induced dose and time-dependent apoptosis in CLL cells, sparing normal B lymphocytes. Furthermore, MI-2 abrogated survival signals provided by stromal cells and BCR cross-linking and was effective against CLL cells harboring features associated with poor outcomes, including 17p deletion and unmutated IGHV. Notably, MI-2 was effective against CLL cells collected from patients harboring mutations conferring resistance to ibrutinib. Overall, our findings provide a preclinical rationale for the clinical development of MALT1 inhibitors in CLL, in particular for ibrutinib-resistant forms of this disease.

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Introduction

Activation of B-cell receptor (BCR) and NF-κB pathways within the lymph node (LN) microenvironment are critical for CLL proliferation and survival (1). High rates of clinical response in patients treated with kinase inhibitors validate the therapeutic targeting of these pathways in CLL (2–6). Ibrutinib covalently binds to and irreversibly inhibits Bruton’s tyrosine kinase (BTK), an essential kinase for BCR signaling (2, 7, 8). Objective response rates with single-agent ibrutinib are as high as 70% in relapsed or refractory patients and can reach 86% in first-line therapy (2, 7). Equally high initial response rates have been reported in patients with deletion 17p (6, 9). However, patients with deletion 17p, especially those with relapsed or refractory disease, have shorter progression-free survival than patients without these high-risk features (10, 11). In fact, more than half of all patients with previously treated CLL enrolled in the phase Ib/II trial suffered disease progression within 5 years on ibrutinib, and patients with 17p deletion had a median progression-free survival of 26 months (12). In the NIH cohort, median progression-free survival was 38.8 months in patients with high-risk disease in either the treatment-naïve or relapsed settings (13). High risk is defined by having a TP53 aberration and a Rai stage III/IV (in previously treated patients) in addition to a β2-microglobulin >4 (in previously untreated patients; ref. 13). The most common findings in patients resistant to ibrutinib are nonsynonymous mutations affecting the cysteine 481 (C481) of BTK, which forms a covalent bond with ibrutinib, and gain-of-function mutations in PLCG2 downstream of BTK. Other less frequent genetic alterations have been associated with ibrutinib resistance, including δP deletion (10, 14–16). Ibrutinib-resistant CLL can progress rapidly and effective treatment options are limited (11, 17, 18). Objective responses following progression on ibrutinib have been observed with the BCL2 antagonist venetoclax and the PI3Kδ inhibitor idelalisib (19).

The paracaspase mucosa-associated lymphoid tissue lymphoma translocation 1 (MALT1) was first identified as the fusion partner in the translocation t(11;18)(q21;q21) found in a subset of mucosa-associated lymphoid tissue (MALT) lymphoma.

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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Targeting MALT1 in CLL

MALT lymphomas typically arise as antigen-driven lymphomas and can remit after an infecting infection is eradicated. The chromosomal translocation creates the API2–MALT1 fusion oncprotein that promotes antigen-independent MALT1 activation and NF-κB signaling (20). MALT1 is the enzymatically active component of the CARD11–BCL10–MALT1 (CBM) signaling complex (21). The gatekeeper role of the CBM complex is also evidenced by CARD11 mutations that are oncogenic drivers in a subset of activated B-cell–like diffuse large B-cell lymphoma (ABC-DLBCL; ref. 22). Furthermore, recurrent gain-of-function germline variants in RNF31, a component of the linear ubiquitin chain assembly complex that cooperates with CBM to activate NF-κB, has been implicated in lymphomagenesis (23).

Given its oncogenic role in lymphomas, targeting MALT1 has been pursued as a therapeutic strategy (24–28). MI-2 (C19H17Cl3N4O3) covalently binds to C464 within the para-caspase domain of MALT1 and thereby suppresses its protease activity (24). Fontan and colleagues showed that MI-2 irreversibly inhibits the cleavage of MALT1 substrates in ABC-DLBCL cell lines, thereby reducing constitutive NF-κB pathway activity and cell proliferation and survival (24). In contrast, germinal center B-cell–like (GCB)-DLBCL, which lacks constitutive NF-κB activation, was not sensitive (24, 29). At least in mice, MI-2 was far so well-tolerated and not associated with specific toxicities (24).

The possible therapeutic role of MI-2 in CLL has not been investigated. We hypothesized that MALT1 inhibition could have antileukemic activity in CLL. Further, given that most mutations associated withibrutinib resistance reactivated NF-κB signaling upstream of MALT1, we hypothesize that targeting MALT1 could be effective in ibrutinib-resistant CLL.

Patients and Methods

Patients and samples
Peripheral blood mononuclear samples (PBMC) were obtained from treatment-naïve and relapsed CLL patients (Supplementary Tables S1 and S2). Written informed consent in accordance with the Declaration of Helsinki was obtained from all Institutional Review Boards at Tulane University (New Orleans, LA; #M0600) and at the NHLBI (NCT00923507). PBMCs were isolated using density gradient centrifugation with lymphocyte separation medium (ICN Biomedicals). Fresh cells were subjected to CD19 selection using magnetic beads yielding purity >96% (Miltenyi Biotec). As previously described, IGHV sequencing was performed on leukemic samples and classified as mutated (<98% homology to germline) or unmutated (>98% homology; ref. 30). In select experiments, we utilized clinical samples collected from patients with CLL treated with single-agent ibrutinib on a phase II trial (NCT01500733) at baseline, samples collected from patients with CLL treated with single-agent ibrutinib on a phase II trial (NCT01500733) at baseline, and single-nucleotide variant callings. Variants were annotated

RNA sequencing and gene expression analysis
Total RNA was extracted from CLL PBMCs using RNeasy kit (Qiagen), and cDNA was prepared using the High Capacity cDNA RT Kit (Applied Biosystems). NF-κB–specific gene signature score was determined as previously described (34). Briefly, expression of six NF-κB target genes was quantified by real-time PCR (RT-PCR) on TaqMan Primers on an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems). The difference in threshold cycle (ΔCt) for each gene of interest was calculated from the Ct of the housekeeping gene (VCP)—Ct of the gene of interest (e.g., CCL3). The ΔCt for the pathway–specific genes (six unique genes for NF-κB) were averaged into a signature score.

Flow cytometry
PBMCs were treated with various concentrations MI-2 or DMSO (control) in the presence or absence of 100 μmol/L of the pan-caspase inhibitor z-VAD-fmk (R&D Systems). Cells were stained with anti–Annexin V, and CD19 (BD Biosciences), ViViD Live-Dead stain, and/or TO-PRO-3 stain (Invitrogen), and analyzed by flow cytometry as previously described (31). In select experiments cells were stained for the activation markers CD69 and CD86 (BD Biosciences).

Immunoblot
Immunoblot experiments were carried out as previously described (32). Primary antibodies used were anti-MALT1 (cat# 2494) and anti-c-PARP (Asp214; cat# 9541), t-PARP (cat# 9532a), GAPDH (cat# 2118S), Bcl-xL (cat# 2764S), all from Cell Signaling Technology; CYLD (cat# sc-137139) and Actin (cat# sc-47778), both from Santa Cruz Biotechnology. Secondary antibodies used were IRDye goat anti-rabbit (cat# 926-68071), IRDye goat anti-mouse (cat# 926-32210), both from LI-COR Biosciences or ECL donkey anti-rabbit IgG HRP (GE Healthcare). The signal was visualized using Odyssey Imaging System (LI-COR Biosciences) or LAS-4000 Imaging System (Fuji Film).

Assessment of the protective effect of the microenvironment
Primary CLL cells were cocultured in the presence or absence of nurse-like cells (NLC) or 5 μg/ml of anti-Human IgM (Jackson ImmunoResearch, cat#109-006-129), with or without MI-2, for 24 hours (33). CLL cell viability was determined by flow cytometry using TO-PRO-3.

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using ANNOVAR. RNA sequencing data are deposited in GEO under accession number GSE98206.

NF-κB activity assay
NF-κB activity was measured using the TransAM NF-κB transcription factor assay kit (Active Motif). CLL PBMC’s were treated with or without MI-2 (2.5 and 5 μmol/L) for 6 hours. Nuclear lysates were extracted via Nuclear Extract Kit (Active Motif). Nuclear lysates were applied to 96-well plates coated with oligonucleotides containing NF-κB consensus sequence (5′-GGGACTTTCC-3′). The change in expression of RelB and p50 isoforms were determined by comparing samples to matched untreated controls.

Droplet digital PCR
Droplet digital PCR (ddPCR) was performed on DNA isolated from stored samples after CD19+ selection, as described previously (36). Briefly, custom ddPCR assays were obtained from Bio-Rad Laboratories and analyzed using a Bio-Rad QX200 droplet reader. Each mutation detection assay was run duplexed to a matched wild-type assay (primer and probe sequences; Supplementary Table S3) in quadruplicates. Variant allele frequencies (VAF) were calculated from the fraction of positive droplets with a reported sensitivity of 0.01%.

Statistical analysis
The Student t test (paired or unpaired). Fisher exact test, and one-way analysis of variance were used to assess the differences between groups. All P values were two-sided, and values <0.05 were considered statistically significant. For RT-PCR data, the raw Ct value was normalized to internal control. Analyses were performed using GraphPad Prism (GraphPad Software Inc.), JMP software (SAS Institute), and R statistical software 3.2.2 (Institute for Statistics and Mathematics).

Results
MALT1 is more active in CLL compared with normal B cells
We first sought to determine the protein expression patterns of MALT1 in primary CLL cells and normal B cells. To this end, we used immunoblot assays to compare MALT1 protein levels in CD19-selected CLL cells collected from 21 patients, and in B cells of six normal volunteers. We found that MALT1 protein expression was highly variable in CLL, and lower than in normal B cells on average (Fig. 1A and B). In order to determine whether MALT1 is active in CLL, we measured cleaved CYLD in freshly isolated primary CLL cells and normal B cells. CYLD is a direct target of MALT1, and the degree of CYLD cleavage (Fig. 2D) and reduced MI-2 potency was detected in normal B cells, indicating that MALT1-dependent proteolysis is ongoing in CLL in vivo, but not in normal B cells (Fig. 1A and C).

Inhibition of MALT1 with MI-2 induced dose- and time-dependent apoptosis in CLL cells
We first evaluated effects of MI-2 in MEC1 cells. MI-2 dose-dependently reduced cell viability, with an inhibitory concentra-

Figure 1.
MALT1 is constitutively active in CLL. A, A representative immunoblot analysis of MALT1, CYLD, and the C-terminal cleaved form of CYLD (CYLD(C24), a 70 KDa CYLD cleavage product) in CD19-selected primary CLL samples and normal B cells. MEC1 and K562 were used as positive and negative controls, respectively. Actin is shown as a loading control. B, MALT1 protein levels shown as CYLDC-ter/Actin in CLL (N = 6) measured by immunoblot as described in A, normalized using MALT1/Actin ratio of MEC1 across different experiments. C, Cleaved CYLD (CYLD(C24)) protein levels shown as CYLD(C24)/Actin in CLL (N = 8) and normal B cells (N = 6) measured by immunoblot as described in A, normalized using CYLD(C24)/Actin ratio of MEC1 across different experiments. Comparisons in B and C were conducted using unpaired Student t test. *P < 0.05.
MI-2 thwarts the protective effect of the microenvironment in CLL

Next, we used the NLC coculture system or anti-IgM-mediated BCR activation to determine whether MI-2 can overcome tumor-microenvironment interactions or antigen stimulation (33, 39). To this end, we treated primary CLL cells cultured in the presence or absence of NLC ($N=5$) or after IgM activation ($N=4$) with MI-2 ($0.5–4\ \mu\text{mol/L}$) for 48 hours. Coculture with NLC or with anti-IgM improved the viability of the untreated primary CLL samples in vitro (Fig. 3A–C). Despite this, MI-2 remained effective against CLL cells in the presence of NLC and BCR crosslinking (Fig. 3A–C). Akin to ibrutinib, we observed a reduction in MI-2 cell-killing ability at low concentrations ($\leq 2\ \mu\text{mol/L}$) in the presence of NLC compared with the no-NLC group (40). However, NLC loses their protective effects when MI-2 is used at 4 $\mu\text{mol/L}$ (Supplementary Fig. S2A). In comparison, BCR crosslinking did not provide any protective effects against MI-2 for any of the concentrations used (Supplementary Fig. S2B). Further, there was no cytotoxic effect on NLC at any of the used concentrations of MI-2 (measured by MTS assay, data not shown).

We next sought to investigate whether disease heterogeneity is a driving factor in MI-2 sensitivity. We characterized our samples for IGHV mutational status, 17p deletion, prior treatment status, and CD38 expression. With the exception of slightly increased sensitivity of IGHV mutated samples at 2.5 $\mu\text{mol/L}$, treatment with MI-2 was equally active against CLL cells regardless of 17p deletion status, prior therapy status, or CD38 expression (Supplementary Fig. S2C–S2F).

MI-2 inhibits NF-κB signaling and disrupts a myriad of biological networks in CLL

In ABC-DLBCL cell lines, 8 hours of MALT1 inhibition effectively inhibited expression of NF-κB-regulated genes (24, 28). To investigate the effect of MI-2 on CLL tumor biology, RNA from purified CLL cells treated for 8 hours with 2 $\mu\text{mol/L}$ MI-2 in vitro ($N=3$) was subject to RNA sequencing and compared with untreated controls ($N=3$). Out of 24,462 tested genes, there were 438 genes whose expression changed 2-fold at $P<0.05$ (312 down- and 126 upregulated; Fig. 4A; Supplementary Table S4).

To identify the biologic basis for the MI-2-induced changes in gene expression, we chose Gene Set Enrichment Analysis (GSEA) as an investigator-independent discovery tool (41). GSEA identified 35 Hallmark and Oncogenic Signatures gene sets that were differentially enriched between the treated and untreated samples at $P<0.05$. The complete list of enriched gene sets is provided in Supplementary Tables S5 and S6.
samples compared with their control counterparts at FDR ≤ 5%, and normalized enrichment score (NES) ≥ 1.50. Of these, 32 gene sets relevant to CLL were grouped based on their functional similarities into two distinct categories (Supplementary Table S5): “Signaling/Interaction with the Microenvironment” including BCR and NF-κB signaling pathways; and “Proliferation/Malignancy,” all were downregulated by MI-2. Collectively, GSEA clearly identified the BCR and NF-κB pathways among the top affected pathways (Fig. 4B and C; Supplementary Table S5). MI-2 inhibits CYLD cleavage, suppresses NF-κB translocation to the nucleus, and restores apoptosis in CLL.

Cleavage of CYLD is ongoing in CLL and represents direct evidence of the proteolytic activity of MALT1 (Fig. 1A and C). To investigate the direct effect of MI-2 on MALT1 proteolytic activity, we measured the effect of MI-2 on CYLD cleavage, and detected a clear reduction in the level of cleaved CYLD (Fig. 5A).

MALT1 has been implicated in both canonical and noncanonical NF-κB signaling (21, 42). To evaluate the impact of MI-2 on canonical and noncanonical NF-κB activation, we used an ELISA to measure the nuclear levels of p50 and RelB, respectively. Indeed, treatment with MI-2 resulted in a statistically significant, dose-dependent reduction of both p50 (Fig. 5B) and RelB (Fig. 5C) nuclear levels. Consistently, we observed decreased expression of the activation markers CD69 and CD86 on the surface of CLL cells following treatment with MI-2 (Fig. 5D). Similarly, the protein level of Bcl-xL, an antiapoptotic protein whose expression is transcriptionally controlled by NF-κB (43), was reduced in cells treated with MI-2 (Fig. 5E). MI-2 is effective against ibrutinib-resistant CLL cells

A treatment-induced lymphocytosis at the start of therapy with kinase inhibitors is well appreciated and does not cause morbidity or predict inferior outcome (11, 44). While most patients have resolution of lymphocytosis within the first year, a subset of patients have persistent lymphocytosis for years raising concerns about the risk of acquired resistance (44). We sought to determine whether MI-2 can overcome “ibrutinib-tolerance” in these cells. We therefore collected PBMCs from CLL patients having an absolute lymphocyte count >10,000 cells/μL at 1 year from the start of ibrutinib and within 4 to 12 hours of their last dose of ibrutinib (on-treatment sample) and compared their drug sensitivity to matched samples obtained before the start of ibrutinib (baseline sample; Supplementary Fig. S3A). The IC50 of MI-2 against on-treatment samples was 2.5 μmol/L, comparable with the IC50 previously determined for ibrutinib-naïve samples (Fig. 6A). In a head-to-head comparison of patient-matched baseline and on-treatment samples, the sensitivity to MI-2 in the on-treatment samples appeared slightly increased compared with baseline (Fig. 6B).

To test the activity of MI-2 in ibrutinib-resistant disease, we exposed PBMCs collected from patients who progressed on...
ibrutinib to increasing concentrations of MI-2 in vitro. These samples were collected either at the time of relapse while remaining on ibrutinib, or after the drug was stopped but prior to initiation of another therapy. CLL cells collected at the time of progression were sensitive to MI-2 treatment (Fig. 6C). As reported, most cases of ibrutinib resistance in our patients were associated with mutations in BTK and/or PLCG2 (10). We found that cells collected from patients with a mutation in either one of these genes (or both) were sensitive to MI-2 with IC50 values comparable with baseline samples (N = 8; Supplementary Fig. S3B). However, cells from patients with an unknown resistance driver tended to be less sensitive to MI-2 (Supplementary Fig. S3B). Where possible, we tested MI-2 effects against samples from patients at baseline, on-treatment, and at progression and found that they were equally sensitive to MI-2 for 24 hours and determined the VAF of mutated BTK (C481S) or PLCG2 (R665W or L845F) in purified tumor cells using a digital PCR assay. Following treatment with MI-2, CLL cells in two out of three patients with a BTK mutation showed a significant reduction in VAF, suggesting that mutated cells were more sensitive to MALT1 inhibition than the cells with wild-type BTK (Fig. 6E). In the third patient, there was no difference. VAFs of PLCG2 mutations were low and did not change with MI-2 treatment, suggesting that both the mutated and unmutated cells responded equally to MI-2 treatment in vitro (Fig. 6E).

Discussion
Progressive CLL develops in about half of high-risk patients during the first 3 years on ibrutinib and is the leading cause of death for these patients (10, 15). Thus, novel therapeutic strategies are needed to regain disease control in patients progressing on...
ibrutinib. Here, we show that the para-caspase MALT1 is a promising target in CLL, particularly for ibrutinib-resistant disease. We show for the first time that MALT1 is constitutively active in CLL, and that targeting MALT1 with MI-2 is effective against ibrutinib-resistant tumor cells. MI-2 was equally effective against CLL cells activated through the BCR or cultured in the presence of NLC or having adverse genetic features such as unmutated IGHV and 17p deletion. Further, MI-2 was selectively toxic for CLL cells, while sparing nonmalignant B cells. Like ibrutinib, MI-2 potently inhibited BCR and NF-κB signaling. In contrast to ibrutinib, MI-2 induced apoptosis, which could result in deeper clinical responses.

MALT1 and the CBM complex connect antigen signaling to NF-κB activation. Oncogenic lesions that activate CBM confer antigen-independent NF-κB activation in lymphoma (20, 22). In CLL MALT1 appears constitutively activated, likely as part of BCR signaling, and is turned off by MI-2. Most known ibrutinib resistance mutations reactivate BCR signaling upstream of
Figure 6.
MI-2 is effective against ibrutinib-resistant CLL. A, Primary CLL PBMCs collected from patients treated for 12 months with ibrutinib were incubated with or without MI-2 at 0–10 μmol/L. Mean ± SEM % viable CLL cells (CD19+/Annexin V−/ViViD−) is shown after 24 hours of MI-2 treatment (N = 10). B, % point difference in cell death of CLL cells from patients on ibrutinib compared with pretreatment is shown after 24 hours exposure to 2.5 μmol/L MI-2 in vitro (N = 8). C, PBMCs collected from CLL patients with acquired ibrutinib resistance were incubated with or without MI-2. % viable CLL cells (CD19+/Annexin V−/ViViD−) is shown after 24-hour exposure to increasing concentrations of MI-2 relative to untreated control (N = 10). Red symbols represent samples with BTK mutations, blue symbols represent PLCγ2 mutations, purple symbols represent samples with both BTK and PLCγ2 mutations, and black symbols represent patients with no known resistance mutations. D, Mean ± SEM % change in viable CLL cells (CD19+/Annexin V−/ViViD−) collected from patients prior to treatment (Pre), on in vivo ibrutinib (ON) and after progressing on ibrutinib (PD) is shown after 24-hour exposure to 2.5 μmol/L of MI-2 relative to untreated control (N = 7). E, VAF in samples from patients shown in C with BTK C481S mutations or PLCγ2 R665W or L845F mutations left untreated or exposed to 5 μmol/L MI-2 for 24 hours (N = 7) is shown. NS, non-significant; **, P < 0.01; ****, P < 0.0001. SEM, standard error of mean.
MALT1. Thus, targeting MALT1 provides an opportunity to overcome the effects of BTK and PLCG2 mutations at the next critical junction in signal transduction. Basal activity of MALT1 in CLL is evident by the higher level of CYLD cleavage detected in comparison with normal B cells. Consistent with increased protease activity, including autoleucylation (45), MALT1 levels are lower in CLL compared with normal B cells. Similar observations were described by Dai and colleagues who detected lower levels of MALT1 and higher levels of cleaved CYLD in mantle cell lymphoma cell lines with active MALT1 compared with cell lines with inactive MALT1 (25). Other MALT1 targets include A20, BCL10, and RELB (21, 28).

Reduced CYLD cleavage and inhibition of NF-κB signaling in primary CLL cells by MI-2 is consistent with data in lymphoma cell lines (24, 25). However, our transcriptome analysis in CLL cells treated with MI-2 revealed not only the expected downregulation of NF-κB target genes but suggested a broader impact of the drug, including inhibition of the JAK-STAT, interferon, and Ras signaling pathways (46, 47). NF-κB inhibition is reflected in decreased levels of nuclear p50 and RelB, and select NF-κB regulated molecules, in particular Bcl-xL. MALT1 has been implicated in both canonical and noncanonical NF-κB signaling, and its absence has been shown to impair B cell activation factor (BAFF)-mediated translocation of RelB into the nucleus in MALT1−/− cells, consistent with our data (42).

Direct induction of apoptosis distinguishes MI-2 from BCR inhibitors, particularly ibrutinib, which promotes little apoptosis when used at high concentrations (48–50). Like ibrutinib, MI-2 was very effective against cells with or without adverse characteristics such as unmuted IGHV and 17p deletion. MI-2 also abrogated the survival support of NLC and anti-IgM crosslinking, microenvironmental stimuli that support CLL survival in vivo and that can promote resistance to chemotherapy as well as novel agents (51, 52). The pan-caspase inhibitor Z-VAD-fmk rescued cells from MI-2–induced cytotoxicity consistent with induction of apoptosis due to MALT1 inhibition. Notably, the MALT1 protease cleaves substrates after Arg, while caspases cleave after Asp, and is not affected by Z-VAD-fmk (21).

Despite recent improvements, the management of progressive disease on BCR inhibitors remains challenging (53). The median survival for patients progressing on ibrutinib with CLL is around 2 years, and even shorter for Richter’s transformation (10, 15, 17). Objective responses with venetoclax following ibrutinib failure have been achieved in 70% (54). However, complete remissions were seen in only 2% (18), likely predicting for short duration of response based on prior studies with venetoclax (54). There is less experience with the PI3Kδ Ilekalisib in this setting, and the overall response rate at 28% is lower (55). While mutations in CARD11 have been associated with ibrutinib resistance in diffuse large B-cell lymphoma, CARD11 mutations are uncommon in CLL. Notably, MI-2 restored sensitivity to ibrutinib in CARD11 mutant cells and induced CARD11 degradation, resulting in NF-κB inhibition (27). Mutations in MALT1 or in elements downstream to MALT1 have not been described as causes of ibrutinib resistance, suggesting that targeting MALT1 could overcome resistance. In agreement, we show that MI-2 remained active in ibrutinib-resistant disease and that subclones harboring mutations in BTK and/or PLCG2 were at least as sensitive as their wild-type counterparts. These results also suggest that MI-2 might overcome resistance to other BTK inhibitors in CLL, such as acalabrutinib whose mechanisms of resistance also appear to be mediated by mutations in BTK and/or PLCG2 (3).

Interestingly, "ibrutinib-tolerant" cells that persisted for 12 months or more in patients on treatment were more sensitive to MI-2 compared with matching cells collected prior to starting ibrutinib, suggesting that the two drugs might act synergistically, providing a rationale to explore concurrent targeting of BTK and MALT1.

In conclusion, these data identify MALT1 as an outstanding therapeutic candidate for ibrutinib-resistant CLL. MALT1 inhibitors should be investigated in clinical trials for patients progressing on BCR inhibitors.

Disclosure of Potential Conflicts of Interest

N.S. Saba has received honoraria from speakers bureau of Pharmacyclics. H. Safah has provided expert testimony as primary investigator on a Pharmacyclics-sponsored clinical trial. A. Melnick reports receiving a commercial research grant from Janssen. A. Wiestner reports receiving commercial research grant from Pharmacyclics and Acerta. No potential conflicts of interest were disclosed by the other authors.

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