Prolonged lymphocytosis during ibrutinib therapy is associated with distinct molecular characteristics and does not indicate a suboptimal response to therapy

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Introduction

Chronic lymphocytic leukemia (CLL) is a common adult leukemia and is currently incurable outside of stem cell transplantation. Although chemoimmunotherapy has improved survival,1,2 patients who relapse have poor outcomes with additional standard therapies. Also, many standard therapies are associated with significant toxicities and sustained immunosuppression.3,4 Identifying effective therapies with better toxicity profiles is thus a high priority, and targeted therapies may allow attainment of this goal.

One broad target is the B-cell receptor (BCR) signaling pathway. In normal B cells, ligation of the BCR results in a signaling cascade that can lead to proliferation, apoptosis, or anergy depending on the stage of development and antigen ligated.5 In CLL cells, however, the BCR is dysregulated, and activation through antigen ligation or auto-stimulation results in the propagation of proliferative and prosurvival signals.6,7 Although multiple agents are in clinical development that target the BCR, one of the most exciting is the Bruton’s tyrosine kinase (BTK) inhibitor ibrutinib. Ibrutinib binds BTK irreversibly at the Cys481 residue in the active site, rendering it kinase inactive. This inhibition has been shown in vitro to induce modest CLL cell apoptosis and to abolish proliferation and BCR signaling.8,9 Clinical trial results with this agent have been outstanding, including an estimated 26-month progression-free survival (PFS) of 75% for patients with relapsed and refractory disease.10

Although PFS with ibrutinib is excellent, the overall response rate for this group of relapsed patients is only 71%,10 lagging behind the clinical benefit seen in 88% of patients because of lymphocytosis induced by this agent and all agents targeting the BCR pathway. BCR-associated lymphocytosis was first recognized with fostamatinib and may be due to disruption of signaling through CXCR4-SDF1 and other adhesion factors in the marrow and nodal sites, leading to cell mobilization.11 Although this phenomenon has been recognized with fostamatinib, idelalisib,12 and now ibrutinib,13 the characteristics of these lymphocytes and the consequences of this lymphocytosis have been unexplored. In this

Key Points

- Persistent CLL cells during ibrutinib therapy show evidence of biochemical activation, but inhibited BCR and no proliferation.
- Long lymphocytosis during ibrutinib therapy is not associated with adverse progression-free survival.

The Bruton’s tyrosine kinase (BTK) inhibitor ibrutinib has outstanding activity in patients with chronic lymphocytic leukemia. Most patients experience lymphocytosis, representing lymphocyte egress from nodal compartments. This resolves within 8 months in the majority of patients, but a subgroup has lymphocytosis lasting >12 months. Here we report a detailed characterization of patients with persistent lymphocytosis during ibrutinib therapy. Signaling evaluation showed that while BTK is inhibited, downstream mediators of B-cell receptor (BCR) signaling are activated in persistent lymphocytes. These cells cannot be stimulated through the BCR and do not show evidence of target gene activation. Flow cytometry for κ and λ expression, IGHV sequencing, Zap-70 methylation, and targeted gene sequencing in these patients are identical at baseline and later time points, suggesting that persistent lymphocytes do not represent clonal evolution. In vitro treatment with targeted kinase inhibitors shows that they are not addicted to a single survival pathway. Finally, progression-free survival is not inferior for patients with prolonged lymphocytosis vs those with traditional responses. Thus, prolonged lymphocytosis is common following ibrutinib treatment, likely represents the persistence of a quiescent clone, and does not predict a subgroup of patients likely to relapse early. (Blood. 2014;123(12):1810-1817)
report, we present the first data regarding the scope of lymphocytosis observed with ibrutinib and a detailed characterization of persistent lymphocytes relative to pretreatment lymphocytes. Also, we will report clinical outcomes associated with these patients to establish the clinical consequences of persistent lymphocytosis with ibrutinib.

Methods

Patient sample processing and cell culture

Blood was obtained from patients with relapsed CLL participating in institutional trials of ibrutinib who had provided informed consent in accordance with the Declaration of Helsinki and under a protocol approved by the Institutional Review Board of The Ohio State University. All patients were treated with ibrutinib at doses of 420 or 840 mg daily and were on continuous therapy when samples were collected. Peripheral blood mononuclear cells were isolated using methods detailed in the supplemental Methods on the Blood Web site. CD19+ cells were not specifically isolated; however, clinical flow cytometry was obtained in all patients at 6 and 12 months during the study. At 6 months, for the 19 patients whose samples were used in the experiments outlined, the average percentage of lymphocytes that were CLL cells was 93% (range, 83-99%), and at 12 months, the average was 88% (range, 72-96%).

Immunoblotting and real-time reverse-transcription–polymerase chain reaction

Whole cell lysates and RNA were prepared as previously described by our group. Nuclear and cytoplasmic lysates were prepared using the NE-PER Nuclear and Cytoplasmic Extraction Reagent Kit (Thermo Scientific, Waltham, MA) according to manufacturer’s instructions. Equivalent amounts of protein were separated on polyacrylamide gels and transferred onto nitrocellulose membranes. After antibody incubations, proteins were detected with chemiluminescent substrate (SuperSignal; Thermo Scientific).

Antibodies against phospho-BTK(Tyr223), phospho-AKT(Ser473), AKT, ERK1/2, phospho-PLCγ2(Tyr785), and PLCγ2 were obtained from Cell Signaling Technologies (Beverly, MA). Phospho-Erk(Thr202/Tyr204) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were purchased from Santa Cruz Biotechnologies (Dallas, TX).

RNA was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer’s directions and converted to cDNA with the SuperScript First-Strand Synthesis System (Invitrogen). Real-time reverse transcription–polymerase chain reaction (RT-PCR) was performed using Taqman primers from Applied Biosystems (Foster City, CA), and was performed on an Applied Biosystems ViiA7 Real Time PCR System to allow for optimal detection with 1 μL of the PCR product and the sequencing primer zap70_us_SEQ 5′-ATGAGTTGAAATTTGG-3′ following the standard Qiagen protocol with Qiagen PyroMark reagents, GE streptavidin Sepharose beads (GE Healthcare, Pittsburgh, PA), and the PyroMark Q96 Vacuum Workstation.

Zap-70 methylation analysis

The methylation of ZAP-70 at CpG319 was assessed by pyrosequencing. Genomic DNA was bisulfite-treated using the Zymo EZ DNA Methylation-GoldKit (Zymo, Irvine, CA). Full details on PCR primers and conditions can be found in the supplemental Methods. The pyrosequencing assay was performed on a PyroMark Q96 MD machine (Qiagen, Gaithersburg, MD) with setting CDTI003 using 8 μL of the PCR product and the sequencing primer zap70_us_SEQ 5′-ATGAGTTGAAATTTGG-3′ following the standard Qiagen protocol with Qiagen PyroMark reagents, GE streptavidin Sepharose beads (GE Healthcare, Pittsburgh, PA), and the PyroMark Q96 Vacuum Workstation.

Statistical methods

Most experiments presented herein were conducted to test for differences in phosphorylation or total protein expression using densitometry, gene expression using RT-PCR, or percent viability of cells in patient samples with prolonged and persistent lymphocytosis, post-treatment with ibrutinib relative to pretreatment. Phosphorylated and total protein measures were log transformed, and differences in expression between the two time points were evaluated using random effects models with repeated measures; some patient samples were measured multiple times, and experiment number nested within the donor patient was included as a random effect. Differences in gene expression were evaluated on the ΔΔCT scale using paired t tests. Results were transformed (2−ΔΔCT) to represent fold changes post-treatment relative to baseline. Differences in percent viability between the two time points were also assessed using paired t tests. For all experiments with testing of multiple targets or agents, type I error was protected by adjusting P values using Holm’s method. Two-sided significance levels were set at α = 0.05.
IGHV (41.2% vs 11.9% for PR/CR patients, class interval [CI]: 4.4-8.1). At 12 months, 17 patients (20%) would be time to lymphocyte normalization was 6.2 months (95% confidence interval [CI]: 4.4-8.1). Prolonged lymphocytosis is common after ibrutinib and is likely to have favorable prognostic markers including a deletion of 13q (70.6% vs 35.7% for PR/CR patients, P = .010) and mutated IGHV (41.2% vs 11.9% for PR/CR patients, P = .015).

### Persistent lymphocytes show evidence of BTK and PLCγ2 inhibition, but up-regulation of downstream mediators of BCR signaling

After 1 month of ibrutinib therapy, active forms of BCR signaling proteins are down-regulated (supplemental Figure 1). To determine whether BCR signaling is altered in persistent lymphocytes, we examined serial samples from patients with prolonged lymphocytosis by immunoblot at baseline and at 6 months after the initiation of ibrutinib. We found that BTK autophosphorylation at Y223 was down-regulated in 8 of 8 patients (Figure 1A), and BTK gene expression was not significantly changed (P = .65; Figure 1B). As well, PLCγ2, the immediate downstream target of BTK, showed diminished phosphorylation at Y759 compared with baseline in 7 of 9 patients (Figure 1C), whereas gene expression remained constant (P = .32; Figure 1D). These data confirm that BTK phosphorylation, a surrogate of kinase activity, and PLCγ2 phosphorylation remain inhibited by ibrutinib in most patients with persistent lymphocytosis.

We then evaluated the activity of ERK and AKT to determine whether the BCR signaling pathway remains inhibited distally. Surprisingly, we found that in 9 of 11 patients, ERK phosphorylation...
increases following ibrutinib (Figure 1E), with an average increase of 3.28-fold from baseline (95% CI: 1.75-6.14; \( P < .01 \)). Also, proximal MEK1/2 shows increased phosphorylation after ibrutinib in 9 of 13 patients (Figure 1F), with an average increase of 2.34-fold from baseline (95% CI: 1.22-4.50; \( P = .02 \)). Similarly, phosphor-
ylated AKT is up-regulated in 6 of 7 patients (Figure 1G), with an average increase of 2.61-fold from baseline (95% CI: 1.12-6.11; \( P = .03 \)). These data suggest that downstream signaling through ERK and AKT may be involved in the survival of these lymphocytes in vivo.

These immunoblot data are from baseline to a 6-month time point due to sample availability; however, we see that relative changes from baseline to 6 months are preserved in 12-month samples in a representative subset of patients (supplemental Figure 2).

In persistent lymphocytes, AKT and ERK continue to have mainly cytoplasmic localization and cannot be activated by proximal stimulation of BCR signaling, but can be activated by CD40L

To determine whether cellular localization of BCR signaling proteins is altered in persistent lymphocytes, we prepared nuclear and cytoplasmic lysates on samples obtained at baseline and during ibrutinib therapy from 4 patients with persistent lymphocytosis. In all patients, phosphorylated BTK is present mainly in the cytoplasm prior to ibrutinib therapy and is significantly decreased or absent in persistent lymphocytes (Figure 2A). Consistent with whole cell lysates, phosphorylated ERK increased in all 4 patients and maintained the same localization pattern present pretherapy (Figure 2B).

Phosphorylated AKT is more abundant in the cytoplasm in all patients, and pAKT increases in 2 of 4 patients (Figure 2C). These results show that, although downstream mediators of BCR signaling are amplified, cellular localization does not change, suggesting that there is not increased nuclear recruitment in these persistent cells.

With the up-regulation of ERK and AKT phosphorylation in persistent lymphocytes, we next investigated whether these cells can be stimulated through the BCR or TLR9, which both require BTK, or through CD40L, which activates downstream of BTK. In samples from 3 patients we stimulated cells obtained at baseline and 9 months into ibrutinib therapy. At baseline, these cells were often unable to be stimulated by IgM or CpG, and we found that persistent lymphocytes are unable to be stimulated by IgM or CpG, but can be stimulated by CD40L (Figure 2D), showing that BTK remains kinase inactive. This indicates that, although cells are unable to be stimulated by BTK-dependent pathways, they remain responsive to stimulation outside of BTK or the BCR.

Persistent lymphocytes do not show significant up-regulation of BCR target genes

Next, we sought to determine whether there are distinct gene expression changes in cells from patients who experience persistent lymphocytosis after ibrutinib. From serial samples, we performed real-time RT-PCR to measure gene expression of proximal signaling molecules and targets of nuclear factor \( \kappa \)B (NF\( \kappa \)B). First, we evaluated gene expression of the components of the BCR signalosome: \( CD79 \), \( SYK \), \( LYN \), and \( FYN \). We saw no significant differences between baseline and 12-month expression (Figure 3A). Next, we
sequencing by Ion Torrent technology in 9 patients. Expression and sequencing of the IGHV in 6 patients and targeted gene
 variability in the gene expression while on ibrutinib therapy, there
 were no consistent differences from baseline in the expression of
 these target genes (Figure 3B and supplemental Table 1). To validate,
 we also performed analysis of protein expression of MCL1, BCL2,
 and XIAP in 4 patients and again did not see consistent changes
 in target expression (supplemental Figure 3). These data suggest
 that, although we see evidence of signaling downstream of BTK,
 transcriptional consequences of this up-regulation are not apparent.

To evaluate whether persistent lymphocytes represent an expanding
 subclone of the patient’s original CLL with different IGHV stereotypy
 or alternate κ/λ use, we performed flow cytometry for κ and λ
 expression and sequencing of the IGHV in 6 patients and targeted gene
 sequencing by Ion Torrent technology in 9 patients. κ/λ staining from
 baseline to the 12-month time point was identical in all patients
 (supplemental Table 2). Similarly, all patients had identical VH gene
 use and percent mutation of the IGHV both prior to ibrutinib and after
 1 year of therapy (supplemental Table 2). Ion Torrent sequencing was
 used to detect mutations in 21 genes either known to be recurrently
 mutated in CLL or of interest with ibrutinib therapy. At 12 months,
 mutations and allelic frequency were compared with baseline, and
 none of the 9 patients had acquired additional mutations with a variant
 frequency of >5%. As well, no mutation at 12 months had a variant
 fraction that varied by >10% from baseline. Thus, these data suggest
 that there has been no global alteration in clonal diversity after
 ibrutinib treatment.

Methylation of Zap-70 at CpG3 has been shown to be an
 epigenetic modifier of Zap-70 transcription and therefore expression
 that serves as a strong biomarker of disease19 and is more re-
 producible than Zap-70 expression as assessed by intracellular flow
 cytometry. To determine whether Zap-70 at CpG3 was differentially
 methylated in persistent lymphocytes, we serially assessed for
 methylation of Zap-70 in 6 patients. In all patients, Zap-70 methyl-
 ation did not change significantly after ibrutinib therapy (supple-
 mental Table 3), showing that epigenetic alteration of Zap-70 is not
 altered in persistent lymphocytes.

Persistent lymphocytes are not actively proliferating and are
 not addicted to one signaling pathway

To determine whether persistent lymphocytes are actively pro-
 liferating, we performed flow cytometry for Ki67 on serial samples
 from 7 patients (supplemental Figure 4 and supplemental Table 4). Not
 surprisingly, Ki67 was low in all patients at baseline (median,
 0.6%; range, 0.2%-3.3%). Ki67 was decreased from baseline in all
 patients at 9 months (median, 0%; range, 0%-0.1%; P < .05),
 demonstrating that persistent lymphocytes are not actively pro-
 liferating and indeed proliferation is inhibited similar to what is seen
 with ibrutinib in vitro and at early time points in vivo.20

One area of interest surrounding the peripheral lymphocytosis
 with ibrutinib is whether this can be mitigated through combination
 therapy. Although combinations of ibrutinib and CD20 antibody
 therapy virtually eliminate lymphocytosis,8,20 this effect is almost
 certainly due to the single agent activity of the antibody and not due
 to a disruption of the underlying biology leading to lymphocytosis. To
determine whether persistent lymphocytes are dependent for
 survival on 1 signaling pathway that could potentially be exploited
 by combination therapy, we took serial samples and exposed these
 cells in vitro to agents targeting the BCR pathway, looking for agents
 that were more cytotoxic after long-term ibrutinib therapy. LYN and
 BTK were targeted using dasatinib, PI3 kinase (p110γ and δ) was
 targeted with IPI-145, PKCδ was targeted with AEB-071, the MAP
 kinase pathway was targeted with the MEK1/2 inhibitor CI-1040,
 and AKT was targeted with MK-2206. Also, the cyclin-dependent
 kinase inhibitor dinaciclib was evaluated. As shown in Figure 4, none
 of these agents showed a differential effect pre- vs post-ibrutinib
 (P > .05 when adjusting for multiple tests; supplemental Table 5).
 Notably, dinaciclib showed significant cytotoxicity at both time
 points, suggesting that this could be a rational partner for com-
 bination therapy.

Prolonged lymphocytosis is not associated with adverse
 outcomes after ibrutinib therapy

Given the minimal change in gene expression profile in persistent
 lymphocytosis and absent proliferation, we hypothesized that per-
 sistent lymphocytosis may have little impact in treatment outcome. This
 would contrast with what is typically observed with chemo-
 immunotherapy where gross disease typically is adversely associated
 with a shortened PFS. We examined PFS using a landmark analysis
 in patients achieving a PR-L at 365 days compared with those

Persistent lymphocytes have no evidence of clonal
diversification, clonal evolution, or differential
Zap-70 methylation

Figure 3. Real-time PCR of BCR pathway–associated genes in persistent
lymphocytes. Patient samples at baseline and 12 months of ibrutinib therapy were
analyzed for proximal BCR genes (A) or alternate BCR target genes
(B). There is no consistent change in gene expression of either
proximal BCR genes or (B) BCR target genes.

This would contrast with what is typically observed with chemo-
immunotherapy where gross disease typically is adversely associated
with a shortened PFS. We examined PFS using a landmark analysis
in patients achieving a PR-L at 365 days compared with those
achieving an objective PR or CR by this time. This landmark analysis was performed at this time point to exclude patients with early death or drug discontinuation. As shown in Figure 5, PFS for patients with PR-L is not inferior to those patients who attain a traditional PR or CR prior to 12 months.

Discussion

In this study of patients who experience prolonged lymphocytosis following ibrutinib treatment, we report for the first time, patient characteristics, a detailed molecular characterization of persistent lymphocytes, and the PFS for patients with PR-L vs PR/CR. We see that prolonged lymphocytosis is relatively common following ibrutinib, and although there is some biochemical evidence of activation of these cells, the persistence of lymphocytosis ultimately does not appear to predict a group of patients likely to relapse.

Our laboratory data demonstrate that the majority of these cells exhibit constitutively active downstream BCR signaling, whereas BTK remains inhibited. Although these downstream pathways may indeed contribute to the survival of these cells, we do not see evidence of up-regulation of target genes and transcription factors implicated in survival and proliferation of CLL, and notably, proliferation is absent in these cells. It is also important that targeting of a single pathway in vitro does not lead to cell death, indicating that these cells are not addicted to a single survival pathway. We therefore postulate that these cells represent those CLL cells that are not dependent on proximal BCR signaling for survival and are thus resistant to ibrutinib-induced apoptosis. These cells do not represent the proliferative compartment, however, so their persistence does not lead to rapid disease progression. Additionally, our serial Ion Torrent analysis demonstrates no evidence of new recurring mutations or alternatively increasing clonal aberrations.

Phenotypically, these cells bear some resemblance to anergic B cells with constitutive phosphorylation of Erk1/2.23 However, the

Figure 4. Persistent lymphocytes are not addicted to a single signaling pathway. To determine whether persistent lymphocytes were dependent on a single signaling pathway for survival, cells at baseline and 9 months from patients with persistent lymphocytosis were treated with various inhibitors of the BCR signaling pathway. AEB-071 was dosed at 1 μM, Dasatinib at 5 μM, MK2206 at 1 μM, IPI-145 at 1 μM, CI-1040 at 1 μM, and Dinaciclib at 1 μM. Dinaciclib was washed out at 2 hours. Annexin V/propidium iodide staining and flow cytometry were used to identify viable cells (Annexin negative/PI negative) after 72 hours. No single drug produced more effective cytotoxicity at a late time point compared with baseline. Dinaciclib, a cyclin-dependent kinase inhibitor, induced robust cytotoxicity both at baseline and at 9 months of ibrutinib therapy.

Figure 5. PFS of patients with persistent lymphocytosis is not inferior to those achieving complete or PR by 12 months. This is a landmark analysis at day 365 comparing patients with PR-L at 12 months vs those with CR/PR at 12 months. There is no statistical difference between these groups, although there is a trend toward improved survival in patients with PR-L.
cells described here also show evidence of constitutive Akt activation, which is not a feature of this group. Despite this, given the benign clinical course of these patients, the phenotype that we describe here also likely represents a subgroup of quiescent anergic cells. Supporting this are recent data showing that primary CLL cells contain intracanal subgroups that vary in the level of IgM responsiveness and that ibrutinib preferentially inhibits the most IgM-responsive cells, suggesting that these persistent lymphocytes may represent the subclone of cells that are the most anergic. Also, our baseline data from patients who develop persistent lymphocytosis demonstrate a lack of response to IgM stimulation, which is a hallmark of molecular anergy and may indicate that patients with an anergic phenotype at baseline are more likely to develop persistent lymphocytosis. However, the question of which patients are more likely to develop persistent lymphocytosis will likely not be answered until ibrutinib is more widely available and we are able to evaluate larger numbers of patients who have persistent vs transient lymphocytosis.

An alternative to the hypothesis that these persistent lymphocytes represent a group of cells that are resistant to ibrutinib-induced apoptosis is that these cells are those that formerly resided in the lymph nodes or bone marrow and thus represent the phenotype of more activated cells because of their former niche. This is unlikely, however, as previous studies demonstrated lymph node excursion as early as 24 hours, yet analysis of signaling at early time points demonstrates reduced ERK phosphorylation (supplemental Figure 1).

Most interesting is the finding that patients with persistent lymphocytosis over 1 year have a similar PFS, with a trend toward a superior outcome compared with those who achieve an objective response within this time frame. Although more patients and longer follow-up will be necessary to validate these findings, these results are significant and worthy of further investigation. This could indicate that baseline characteristics that predict a favorable response are the same as those that predict persistent lymphocytosis. Alternatively, the persistence of a quiescent CLL clone may inhibit resistant subclone formation or expansion. At this time, combination therapies with ibrutinib are under investigation with the goal of hastening response and improving the rate of CR. The data presented here, however, suggest that these goals are not necessarily relevant. Although there is convincing evidence that the attainment of a CR and minimal residual disease negativity is important for long-term PFS with chemotherapy, with ibrutinib the elimination of MRD and even gross disease may not be necessary.

These findings also highlight a need to revise consensus response criteria in CLL. The preliminary success of ibrutinib in this disease is likely to lead to a paradigm shift toward targeted small molecules. The BCR pathway is now a validated target in this disease, and because we have seen that lymphocytosis is a class effect, it is very likely that more agents will come into clinical trials that also induce lymphocytosis. As these agents become more widely available and even standard, it is important that physicians understand that isolated progression of lymphocytosis, even when persistent for many months, is not a sign of disease progression, to prevent unnecessary drug discontinuation.

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Authorship

Contribution: J.A.W., G.L., A.J.J., and J.C.B. designed and performed research, analyzed data, and wrote the manuscript; K.S., L.L.S., A.L., Y.Z., R.M., and K.W. performed research; D.L., W.Z., L.R., E.G., and T.J.K. designed and performed research and analyzed data; J.J., J.F., K.M., S.O., R.R.F., and J.C.B. contributed patients, collected data, and performed research; D.F.J. contributed aggregate data, collected data, and analyzed data; and A.S.R. and F.C. analyzed data.

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References


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