BTK^{C481S}-Mediated Resistance to Ibrutinib in Chronic Lymphocytic Leukemia

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ABSTRACT

Purpose

Therapeutic targeting of Bruton tyrosine kinase (BTK) with ibrutinib in chronic lymphocytic leukemia has led to a paradigm shift in therapy, and relapse has been uncommon with current follow-up. Acquired mutations in *BTK* and *PLCG2* can cause relapse, but data regarding the prevalence and natural history of these mutations are limited.

Patients and Methods

Patients accrued to four sequential studies of ibrutinib were included in these analyses. Deep sequencing for *BTK* and *PLCG2* was performed retrospectively on patients who experienced relapse and prospectively on a screening population.

Results

With a median follow-up time of 3.4 years, the estimated cumulative incidence of progression at 4 years is 19% (95% CI, 14% to 24%). Baseline karyotypic complexity, presence of del(17)(p13.1), and age less than 65 years were risk factors for progression. Among patients who experienced relapse, acquired mutations of *BTK* or *PLCG2* were found in 85% (95% CI, 71% to 94%), and these mutations were detected an estimated median of 9.3 months (95% CI, 7.6 to 11.7 months) before relapse. Of a group of 112 patients examined prospectively, eight patients have experienced relapse, and all of these patients had acquired resistance mutations before relapse. A resistance mutation was detected in an additional eight patients who have not yet met criteria for clinical relapse.

Conclusion

Relapse of chronic lymphocytic leukemia after ibrutinib is an issue of increasing clinical significance. We show that mutations in *BTK* and *PLCG2* appear early and have the potential to be used as a biomarker for future relapse, suggesting an opportunity for intervention.

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INTRODUCTION

The irreversible Bruton tyrosine kinase (BTK) inhibitor ibrutinib has transformed therapy for chronic lymphocytic leukemia (CLL). BTK is a Tec family kinase that is critical for the propagation of B-cell receptor signaling. BTK is upregulated in CLL cells as compared with normal B cells, and targeting BTK, both in vitro and in vivo, decreases proliferation and survival signals.¹⁻³ The importance of kinase functional BTK in progression of CLL and its viability as a therapeutic target were further elucidated in human clinical trials.⁴ Notably, ibrutinib was found to have an overall survival advantage compared with

several standard-of-care therapies,^{5,6} leading to its approval by the US Food and Drug Administration for marketing in 2014 for patients who had received at least one prior therapy and in 2016 for untreated CLL.

Ibrutinib has changed the treatment paradigm in CLL and shown the potential for targeted irreversible inhibitor therapies. Although ibrutinib produces durable remissions in the majority of patients, some patients do experience relapse with Richter transformation or progressive CLL.^{7,8} It has been shown previously that relapse on ibrutinib can be a result of acquired mutations in *BTK* at the binding site of ibrutinib or in *PLCG2*, the protein immediately downstream of BTK.⁷ The

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BTK^{C481S} mutation renders ibrutinib a reversible inhibitor with decreased *BTK* binding affinity.⁷ Initial studies in cell lines and primary cells containing the mutant form of *BTK* showed ibrutinib resistance, demonstrated by persistent phosphorylation of *BTK* and enhanced calcium flux activity after drug exposure.^{7,9,10} The described *PLCG2* mutations allow for persistent activation of the B-cell receptor signaling pathway despite inhibition of *BTK*, with enhanced activity in the mutant cells following B-cell receptor ligation.¹¹ Notably, the patients who experience relapse after ibrutinib with Richter transformation and CLL have a poor outcome,^{8,13} often with rapid disease progression after cessation of therapy. Although these mutations have been described in CLL and studies have shown an association between these mutations and ibrutinib resistance,⁸ the numbers have remained relatively small, and the true scope of relapse on ibrutinib and the association with resistance mutations has previously been unknown. Herein, we describe the natural history of ibrutinib-resistant CLL with respect to outcomes and mechanisms of relapse and provide strategies for monitoring resistance mutations unique from other cancer therapeutics.

PATIENTS AND METHODS

Patients

All patients at The Ohio State University (OSU) Comprehensive Cancer Center (Columbus, OH) who were enrolled onto four Institutional Review Board–approved sequential trials of ibrutinib in patients with CLL were included in this analysis. OSU 10032/Pharmacoclytics (PCYC) 1102 (ClinicalTrials.gov identifier: NCT01105247) was a multi-institutional phase IB/II study of single-agent ibrutinib in patients with relapsed, refractory, or treatment-naïve CLL. OSU 10053/PCYC 1109 (NCT01217749) was a single-institution phase II study of ibrutinib in combination with ofatumumab in patients with relapsed or refractory CLL. OSU 11133 (NCT01589302) was a single-institution phase II study of single-agent ibrutinib in patients with relapsed or refractory CLL. OSU 12024/RESONATE (NCT01578707) was a multi-institutional phase III study of single-agent ibrutinib versus ofatumumab in patients with relapsed or refractory CLL. From this study, all patients initially assigned to ibrutinib or who crossed over to ibrutinib after progression with ofatumumab were included at that time.

Targeted Deep Sequencing

BTK and *PLCG2* were analyzed with the use of the Ion Torrent Platform from Life Technologies (Carlsbad, CA). Details regarding sample preparation, library generation, and sequence analysis are provided in the Data Supplement.

Statistical Analysis

Time to discontinuation of ibrutinib therapy was measured from the first date of treatment until the off-study date, censoring patients who had not discontinued ibrutinib therapy at the date of last contact; patients who went off study for transplantation ($n = 5$) or who continued treatment elsewhere ($n = 9$) were censored at that time. Fine and Gray models of cumulative incidence were fit using forward selection to identify variables most strongly associated with discontinuing ibrutinib therapy as a result of transformation or CLL progression. Discontinuation of therapy for other adverse reasons, such as severe toxicity, was accounted for in the models as a competing risk. Variables considered for model inclusion were age, sex, number of prior therapies, baseline lactate dehydrogenase level, fluorescent

in situ hybridization abnormalities [del(17)(p13.1), del(11)(q22.3), trisomy 12, or del(13)(q14)], *MYC* abnormalities, *BCL6* abnormalities, complex karyotype ($\geq$ three abnormalities), and *IGHV* mutational status. All models adjusted for monotherapy with ibrutinib versus combination therapy with ibrutinib and ofatumumab, regardless of statistical significance. When the failure type was CLL progression, potentially important prognostic variables failed to meet the proportional hazards assumption as a result of few events occurring early in the study. For this reason, a landmark analysis at 1 year was performed, including only patients who remained on ibrutinib therapy at that time ($n = 247$); only two CLL progressions had occurred before the landmark time and were excluded from the analysis. Because the hazard ratios from the landmark analysis better represent the risk of CLL progression during the time frame when progressions occurred and because the final proportional hazards models when using all data or a subset of the data in the landmark analysis included the same variables, only results from the landmark analysis are reported.

Table 1. Baseline Clinical Characteristics

Characteristic	No. of Patients (%; N = 308)
Study	
OSU 10032	50 (16)
OSU 10053	71 (23)
OSU 11133	150 (49)
OSU 12024	37 (12)
Age, years	
Median	65
Range	26-91
Sex	
Male	217 (70)
Female	91 (30)
Rai stage	
0	11 (4)
I/II	91 (30)
III/IV	206 (67)
No. of prior therapies	
Median	3
Range	0-16
LDH, U/L	
Median	218
Range	96-1485
BCL6 abnormality on baseline FISH	
No	276 (91)
Yes	27 (9)
MYC abnormality on baseline FISH	
No	238 (79)
Yes	65 (21)
Trisomy 12 on baseline FISH	
No	251 (83)
Yes	52 (17)
Del(13q) on baseline FISH	
No	146 (48)
Yes	157 (52)
Del(11q) on baseline FISH	
No	220 (73)
Yes	83 (27)
Del(17p) on baseline FISH	
No	182 (60)
Yes	121 (40)
Complex cytogenetics at baseline	
No	123 (42)
Yes	172 (58)
<i>IGHV</i>	
Mutated	55 (20)
Unmutated	219 (80)

Abbreviations: FISH, fluorescent in situ hybridization; LDH, lactate dehydrogenase.

Fig 1. Cumulative incidence of ibrutinib discontinuation for transformation or chronic lymphocytic leukemia (CLL) progression. Discontinuation as a result of Richter transformation tended to occur early in ibrutinib therapy, with few transformations after 2 years. In contrast, CLL progression occurred later, with rare progressions occurring before 1 year. Other events, which include infection, other toxicity, comorbidities, and patient or physician choice, tended to occur at a fairly steady rate for the first 3 years and then plateau.

In the subgroups of patients who discontinued therapy as a result of transformation or CLL progression, survival was calculated from the off-study date until the date of death from any cause, censoring patients at last contact. Survival estimates were calculated using the Kaplan-Meier method, and differences between subgroups were tested using the log-rank test. In the subgroup of patients who experienced relapse with *BTK* or *PLCG2* mutations and had serial samples available before relapse, a Kaplan-Meier estimate of the median time to first detection from date of relapse was obtained using interval censoring because a clone of resistant cells was known to develop between two sample submission dates.

A two-sided $\alpha = .05$ was used to declare statistical significance. All statistical analyses were performed using either TIBCO Spotfire S+ Version 8.2 (TIBCO Software, Palo Alto, CA) or SAS statistical software Version 9.4 (SAS Institute, Cary, NC).

RESULTS

Rate of CLL Progression on Ibrutinib Increases With Extended Follow-Up

All 308 patients treated with ibrutinib on four sequential clinical trials at OSU were included in this analysis, with data locked as of June 14, 2016. This includes 237 patients treated with single-agent ibrutinib on three clinical trials^{5,14,15} and 71 patients treated with ibrutinib in combination with ofatumumab.¹⁶ Clinical characteristics are listed in Table 1. Patients had high-risk genetic features; 80% had *IGHV* unmutated disease, 58% had complex stimulated karyotype, and 40% had del(17)(p13.1). Eight patients were previously untreated, and all of these patients were age 65 years or older. Overall, patients had a median of three prior therapies (range, zero to 16 therapies). With a median follow-up time of 3.4 years (range, 0.3 to 5.9 years), 136 patients (44%) remain on therapy, 14 patients (4.5%) have received transplantation or therapy elsewhere, and 158 patients (51%) have discontinued ibrutinib. Among the 158 patients who have discontinued ibrutinib, 83 patients (52.5%) discontinued as a result of disease progression, classified as transformation (including Richter transformation [$n = 27$] and prolymphocytic leukemia [$n = 1$]) or progressive CLL ($n = 55$). An additional 75 patients (24%) discontinued treatment as a result of adverse events, including 31 patients with infection. Of the patients with infection, 21 died within 30 days of the infection. For these patients with fatal infections, 12 had pneumonia, seven had unspecified sepsis, and two had *Pseudomonas* bacteremia. The median time on ibrutinib before discontinuation was 98 days. Transformation tended to occur within the first 2 years of therapy and CLL progressions tended to occur later, with an estimated cumulative incidence of CLL progression at 1 year of 0.7% (95% CI, 0% to 1.6%), which increased to an estimated incidence of 19.1% (95% CI, 13.9% to 24.3%) at 4 years (Fig 1); discontinuation for adverse reasons other than transformation or CLL progression seemed to reach a plateau after 3 years (Fig 1).

We have reported baseline risk factors for transformation, CLL progression, and other adverse reasons at an earlier follow-up,⁸ but with longer follow-up, we can now make stronger conclusions. Of the variables considered, complex karyotype at baseline was the variable most strongly associated with risk for transformation; although the variable of *MYC* abnormalities was the next strongest variable associated with risk for transformation,

Table 2. Multivariable Models for Cumulative Incidence of Ibrutinib Discontinuation According to Reason for Discontinuation

Variable	Transformation		Progressive CLL*		Other Event	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Complex karyotype (yes v no)	5.00 (1.51 to 16.52)	.008	2.81 (1.34 to 5.88)	.006	—	—
<i>MYC</i> abnormality (yes v no)	2.15 (1.00 to 4.65)	.051	—	—	—	—
Del(17)(p13.1) present on FISH (yes v no)	—	—	2.14 (1.15 to 3.96)	.016	—	—
Age ($\geq$ v < 65 years)	—	—	0.49 (0.27 to 0.91)	.023	2.02 (1.25 to 3.28)	.004
Prior therapies > 3 (yes v no)	—	—	—	—	1.99 (1.23 to 3.23)	.005

NOTE. All models were adjusted for treatment with ibrutinib monotherapy versus combination therapy with ibrutinib and ofatumumab.

Abbreviations: CLL, chronic lymphocytic leukemia; FISH, fluorescent in situ hybridization; HR, hazard ratio.

*Landmark analysis at 1 year.

Fig 2. Survival after ibrutinib discontinuation. Patients with disease progression while on treatment with ibrutinib were observed from the time of ibrutinib discontinuation until death. Patients who discontinued therapy as a result of transformation had a median survival of 3.9 months, and patients who discontinued therapy as a result of progressive chronic lymphocytic leukemia (CLL) had a median survival of 22.7 months.

it did not reach statistical significance ($P = .051$; Table 2; Data Supplement). Because few CLL progressions occurred before 1 year, the test for proportional hazards was violated for potentially important prognostic variables (eg, age and *IGHV* status; Data

Supplement) when using all data across all time points. Therefore, a multivariable model was constructed using a landmark analysis at 1 year. Complex karyotype at baseline, presence of *del(17)(p13.1)* on fluorescent in situ hybridization, and age less than 65 years were all independently associated with a risk for CLL progression (Table 2; Data Supplement). Importantly, for patients age ≥ 65 years without complex karyotype or *del(17)(p13.1)* ($n = 52$), the risk of CLL progression was extremely low, with an estimated cumulative incidence of progression of 1.9% (95% CI, 0% to 5.7%) at 4 years. Conversely, for patients younger than age 65 with both complex karyotype and *del(17)(p13.1)* ($n = 46$), the estimated cumulative incidence of progression at 4 years was 44% (95% CI, 26% to 61%). Independent risk factors for ibrutinib discontinuation for adverse reasons other than transformation or CLL progression include older age and higher number of prior therapies (Table 2; Data Supplement). Median survival time from ibrutinib discontinuation was 3.9 months (95% CI, 2.0 to 10.1 months) for patients with transformation and 22.7 months (95% CI, 13.5 months to not reached; Fig 2) for patients with CLL progression.

CLL Progression on Ibrutinib Is Associated With Acquired Mutations in *BTK* or *PLCG2*, Which Can Be Detected Before Relapse

Forty-six patients with progressive CLL had samples at relapse available for deep sequencing by Ion Torrent. Of these, 40 patients

Fig 3. Ibrutinib resistance mutations can be detected before clinical relapse. For 20 patients with a detectable mutation in *BTK* or *PLCG2* at time of relapse, samples before relapse were analyzed retrospectively to determine the interval of time between mutation detection and clinical relapse. An initial clone could be detected at an estimated median of 9.3 months before relapse. UPN, unique patient number.

(87%; 95% CI, 74% to 95%) had mutations in *BTK* and/or *PLCG2* at the time of clinical relapse (Data Supplement). Distribution of mutations included patients with *BTK* C481 mutation only ($n = 31$), mutation in *PLCG2* at described hotspots¹¹ only ($n = 3$), and mutations in both *BTK* and *PLCG2* ($n = 6$). Variant allele frequency was varied and generally correlated with the presence of disease progression in the peripheral blood versus primarily nodal relapse. For the seven patients with variant allele frequency of less than 10%, all but one had disease progression only in the lymph nodes, with low lymphocyte count in the peripheral blood.

For 20 patients who had acquired *BTK* or *PLCG2* mutations, serial samples were available before relapse and were analyzed retrospectively for mutations. Among these patients, relapse was detected incidentally in two patients, by bone marrow biopsy in one patient, by increasing lymphocytosis alone in four patients, by

lymph node enlargement alone in eight patients, and by both lymphocytosis and enlarging nodes in five patients. A clone of resistant cells could first be detected a median of 9.3 months before clinical relapse (95% CI, 7.6 to 11.7 months; Fig 3), occurring as close as 3 months or greater than 18 months before clinical relapse.

Clones of Ibrutinib-Resistant Cells Can Be Detected Prospectively Before Clinical Relapse

On the basis of the finding that patients who experienced relapse on ibrutinib often had rapid progression and poor outcomes, we initiated a clinical-grade mutation monitoring strategy in our institutional Clinical Laboratory Improvement Amendments–certified molecular laboratory starting in November 2014 (Data Supplement). Mutational analysis of the entire coding regions of *BTK*

Fig 4. Sequential monitoring of *BTK* mutation levels. *BTK/PLCG2* sequencing was performed every 3 months using the clinical-grade sequencing assay. In the eight patients with *BTK*^{C481S} detected at greater than 1% variant allelic frequency (VAF) who had not experienced clinical relapse, VAF did increase in all patients.

and *PLCG2* was performed on a cohort of 112 patients every 3 months prospectively. To date, eight patients have experienced clinical relapse, and all eight patients had *BTK*^{C481S} mutations with expansion of the clone before relapse. *BTK*^{C481S} mutations of greater than 1% allelic frequency were detected in an additional eight patients. All but one patient who discontinued therapy and went to hospice without clinical relapse have had increasing circulating CLL cells in the peripheral blood by flow cytometry, and all have had expansion of the resistant clones (Fig 4). Four of these seven patients have increasing lymph node size on computed tomography scan, but none have yet met criteria for clinical relapse. No patients in this cohort have experienced clinical relapse, and no early signs of progression have been noted in patients without *BTK*^{C481S} clones.

DISCUSSION

We and others have previously identified mutations in *BTK* and *PLCG2* as one mechanism of resistance to ibrutinib in CLL. With a large cohort of patients, we demonstrate definitively that these mutations are the predominant mechanism by which CLL becomes resistant to ibrutinib. Perhaps most significantly, we show that clinical resistance is preceded by a prolonged period of asymptomatic clonal expansion, which suggests the ability to pre-emptively target these cells with alternative therapies in the context of a clinical trial before the patient becomes acutely ill with refractory disease. Particularly for high-risk patients in whom allogeneic stem-cell transplantation is often considered and debated as appropriate, such monitoring offers the opportunity to transition these patients to this modality when early signs of resistance are identified. Although data regarding the best approach to these patients are not yet available, this is certainly an area of active investigation.

We show here that both complex karyotype and del(17)(p13.1) are associated with disease progression on ibrutinib, suggesting that markers that predict poor prognosis with standard therapies also confer risk with novel therapies, albeit a delayed risk. Notably, we also show that age is an important risk factor, with younger patients being more likely to experience relapse than older patients. The reason for this is unknown but could be related to disease features or drug features. Younger patients may have a different disease biology that predisposes them to relapse, or alternatively, differences in drug metabolism with aging may allow higher drug exposures and therefore higher *BTK* occupancy in older patients, making them less likely to develop resistance mutations. In addition, biochemical properties of the tumor cells may be different based on age. Further studies will be required to elucidate the mechanism behind this finding.

Our data show that relapse can occur with varied allelic frequencies of mutant cells in the peripheral blood. In our series, low allelic frequencies were generally associated with progression in the lymph nodes only without corresponding peripheral-blood progression. This suggests that progression might be associated with a compartmental acquisition of mutations, with the bulk of the mutated cells present in the lymph node compartment, although this would need to be validated by lymph node biopsies in a cohort of patients. In addition, mutations at low allelic frequencies in the presence of peripheral-blood disease suggest that

cooperating mutations might be important, and further studies into the clonal dynamics of progression are warranted. Because we know that relapse on ibrutinib can cause an accelerated-phase disease, especially when ibrutinib is discontinued,⁸ the finding that a period of asymptomatic mutant clonal expansion precedes relapse is of critical importance. We show here that targeted sequencing for these mutations in the peripheral blood is feasible and may be an effective biomarker to predict relapse in asymptomatic patients. One therapeutic option that is currently under active investigation is adding a second agent to ibrutinib as soon as a mutant clone is detected to prevent clonal expansion. In addition, in select patients with an identified donor, this period of asymptomatic clonal expansion may allow for stem-cell transplantation, although additional cytoreductive therapy before transplantation may be needed. Because of the quick progression upon clinical relapse and poor outcomes, a biomarker of impending relapse that can be easily measured may well be practice changing, although these data will need to be confirmed in other series.

These data show that ibrutinib resistance in patients with high-risk, previously treated CLL is a problem of increasing clinical significance with potentially devastating consequences. Because ibrutinib has been approved by the US Food and Drug Administration for use in the relapsed setting for 2 years, we are likely just starting to see the first emergence of relapse in the community setting. Enhanced knowledge of both the molecular and clinical mechanisms of relapse may allow for strategic alterations in monitoring and management that could change the natural history of ibrutinib resistance.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at jco.org.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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