Original Investigation

Etiology of Ibrutinib Therapy Discontinuation and Outcomes in Patients With Chronic Lymphocytic Leukemia

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IMPORTANCE The Bruton tyrosine kinase (BTK) inhibitor ibrutinib is effective in patients with chronic lymphocytic leukemia (CLL). Reasons for discontinuing therapy with this drug and outcomes following discontinuation have not been evaluated outside of clinical trials with relatively short follow-up.

OBJECTIVE To determine features associated with discontinuation of ibrutinib therapy and outcomes.

DESIGN, SETTING, AND PARTICIPANTS A total of 308 patients participating in 4 sequential trials of ibrutinib at The Ohio State University Comprehensive Cancer Center were included. These clinical trials accrued patients included in this analysis from May 2010 until April 2014, and data were locked in June 2014.

MAIN OUTCOMES AND MEASURES Patients were evaluated for time to therapy discontinuation, reasons for discontinuation, and survival following discontinuation. For patients who discontinued therapy because of disease progression, targeted deep sequencing was performed in samples at baseline and time of relapse.

RESULTS With a median follow-up of 20 months, 232 patients remained on therapy, 31 had discontinued because of disease progression, and 45 had discontinued for other reasons. Disease progression includes Richter's transformation (RT) or progressive CLL. Richter's transformation appeared to occur early and CLL progressions later (cumulative incidence at 12 months, 4.5% [95% CI, 2.0%-7.0%] and 0.3% [95% CI, 0%-1.0%], respectively). Median survival following RT was 3.5 months (95% CI, 0.3-6.0 months) and 17.6 months (95% CI, 4.7 months–"not reached") following CLL progression. Sequencing on peripheral blood from 8 patients with RT revealed 2 with mutations in *BTK*, and a lymph node sample showed no mutations in *BTK* or *PLCG2*. Deep sequencing on 11 patients with CLL progression revealed *BTK* or *PLCG2* mutations in all. These mutations were not identified before treatment in any patient.

CONCLUSIONS AND RELEVANCE This single-institution experience with ibrutinib confirms it to be an effective therapy and identifies, for the first time, baseline factors associated with ibrutinib therapy discontinuation. Outcomes data show poor prognosis after discontinuation, especially for those patients with RT. Finally, sequencing data confirm initial reports associating mutations in *BTK* and *PLCG2* with progression and clearly show that CLL progressions are associated with these mutations, while RT is likely not.

TRIAL REGISTRATIONS clinicaltrials.gov Identifiers: NCT01105247, NCT01217749, NCT01589302, and NCT01578707

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Chronic lymphocytic leukemia (CLL) is the most prevalent leukemia in adults and is not considered curable outside of allogeneic stem cell transplantation. Significant advances have been made in the therapy, notably the emergence of kinase inhibitors for patients with relapsed disease. Prior to US Food and Drug Administration (FDA) approval of ibrutinib and idelalisib with rituximab, standard therapy for relapsed CLL could be expected to induce response rates of 30% to 50%[1-3] and progression-free survival (PFS) of generally less than 1 year. The phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3 kinase) delta isoform inhibitor idelalisib[4,5] and Bruton tyrosine kinase (BTK) inhibitor ibrutinib have significantly improved the outcomes for these patients.

BTK is a critical kinase in the B-cell receptor signaling pathway. This pathway is amplified in CLL and results in amplification of proliferation and antiapoptotic signals.[6-9] By inhibiting BTK, ibrutinib eliminates the activation of these prosurvival pathways[8] and microenvironment survival signals.[8,10] In patients, this translates into high clinical response rates and durable remissions. The phase 1 trial of ibrutinib in relapsed B-cell malignant neoplasms showed activity in a variety of diseases,[11] and the benefit in CLL has been confirmed in phase 1b/2[12,13] and phase 3 trials[14] of ibrutinib as a single agent. In the phase 3 study (RESONATE trial), ibrutinib was compared with ofatumumab. With a median follow-up of 9.4 months, the overall response rate (ORR) was superior with ibrutinib, but more importantly both progression-free survival (median, 8.1 months for ofatumumab vs "not reached" for ibrutinib) and overall survival (OS) (12-month estimates: 81% for ofatumumab and 90% for ibrutinib) were significantly improved with ibrutinib.[14] The OS benefit is particularly impressive considering that follow-up was short, and 14 months following the beginning of enrollment, patients whose disease progressed with ofatumumab therapy were allowed to cross over to ibrutinib therapy.

Ibrutinib has also been studied in combination with chemotherapy and immunotherapy. While laboratory data suggested that combination with CD20 antibodies may be less effective because of interleukin 2–inducible T-cell kinase (ITK) inhibition,[15] which impairs the natural killer cell antibody-dependent cellular cytotoxicity,[16] trials of ibrutinib in combination with rituximab[17] and ofatumumab[18] have shown similarly impressive efficacy. It is yet to be determined whether a combination of ibrutinib with another active agent is superior to ibrutinib as a single agent. While the response rate to ibrutinib has been high, with therapy well-tolerated overall, some patients have experienced relapse, while others have been taken off therapy because of toxic effects or other reasons. Our group and others have shown that relapse in CLL can be mediated by at least 2 separate mechanisms.[19] One is by mutations in *BTK*, which both decrease ibrutinib's affinity for BTK and change the binding from irreversible to reversible. The other is through a variety of mutations in *PLCG2*, the immediate downstream target of BTK. We have identified a number of mutations in *PLCG2* that potentially have gain-of-function effects and likely serve to allow the cell an ability to signal in the presence of ibrutinib. Herein, we describe the characteristics of patients who discontinue ibrutinib therapy for relapse and nonrelapse reasons, identify factors that may contribute to discontinuation, perform deep sequencing for known resistance mutations, and describe outcomes of patients after ibrutinib therapy discontinuation.

At a Glance

- Risk factors for discontinuation of ibrutinib therapy due to reasons other than progressive disease include older age and greater number of prior therapies.
- Risk factors for disease progression include *BCL6* abnormalities on pretreatment fluorescence in situ hybridization and complex karyotype.
- Disease progression on ibrutinib therapy includes Richter's transformation (RT), which tends to occur early in treatment, and chronic lymphocytic leukemia (CLL) progression, which tends to occur later.
- Patients who progress with RT have a median survival of 3.5 months after progression.
- Acquired mutations in *BTK* and *PLCG2* account for most CLL progressions during ibrutinib therapy.

Methods

Patients, Treatment Regimens, and Definitions of Clinical End Points

All patients at The Ohio State University (OSU) Comprehensive Cancer Center who provided written informed consent and were enrolled in 4 sequential trials of ibrutinib in patients with CLL, approved by the institutional review board of OSU, were included in this analysis. Study designs are outlined in the eTable 1 in the Supplement. OSU 10032 (Pharmacyclics Inc [PCYC] 1102; NCT01105247) was a multi-institutional phase 1b/2 study of ibrutinib as a single agent in patients with relapsed/refractory or treatment-naïve CLL.[12] OSU 10053 (PCYC 1109; NCT01217749) was a single-institution phase 1b/2 study of ibrutinib in combination with ofatumumab in patients with relapsed/refractory CLL.[18] OSU 11133 (NCT01589302) was a single-institution phase 2 study of ibrutinib as a single agent in patients with relapsed/refractory CLL.[13] OSU 12024 (NCT01578707 [RESONATE; a trademarked trial name by PCYC]) was a multi-institutional phase 3 study of ibrutinib as a single agent vs ofatumumab in patients with relapsed/refractory CLL.[14] From this study, all patients initially assigned to ibrutinib therapy or who crossed over to ibrutinib therapy following progression of disease with ofatumumab were included.

Ion Torrent Deep Sequencing

DNA was extracted from cryopreserved cells using QIAmp DNA Mini kit (Qiagen). *BTK* (OMIM 300300) and *PLCG2* (OMIM 600220) genes were analyzed using the Ion Torrent platform from Life Technologies. Details regarding library generation and sequencing can be found in the eMethods in the Supplement.

Statistical Considerations

Time to discontinuation of treatment was measured from the first date of treatment with ibrutinib until the off-study date, censoring patients who had not discontinued ibrutinib therapy at the

Table 1. Clinical Characteristics for All Patients

Characteristic	Total (N = 308)
Study, No. (%)	
OSU-10032 (PCYC 1102; NCT01105247)[12]	50 (16)
OSU-10053 (PCYC 1109; NCT01217749)[18]	71 (23)
OSU-11133 (NCT01589302)[13]	150 (49)
OSU-12024 (NCT01578707 [RESONATE])[14]	37 (12)
Age, median (range), y	65 (26-91)
Male, No. (%)	217 (70)
Rai stage, No. (%)[22]	
0	11 (4)
I	69 (22)
II	22 (7)
III	44 (14)
IV	162 (53)
Prior therapies, median (range), No.	3 (0-16)
LDH, median (range), U/L	218 (96-1485) [8 unknown]
Abnormal FISH results, No. (%)[a]	
Del(17p)	113 (37)
Del(11q)	80 (26)
Trisomy 12	53 (17)
Del(13q)	156 (51)
MYC abnormality	64 (21)
BCL6 abnormality	26 (9) [3-6 unknown]
Complex cytogenetics (≥3 abnormalities), No. (%)	169 (58) [15 unknown]
IGHV unmutated, No. (%)	219 (80) [34 unknown]

Abbreviations: FISH, fluorescence in situ hybridization; LDH, lactate dehydrogenase; OSU, Ohio State University; PCYC, Pharmacyclics Inc; RESONATE, a trademark trial name by PCYC.

[a] Abnormal FISH results are not mutually exclusive.

date of last contact; patients who went off study for transplant or continued treatment elsewhere (n = 7) were censored at that time. Median follow-up was calculated among all patients (n = 232) censored for time to discontinuation of treatment. Fine and Gray models of cumulative incidence[20,21] were fit to identify variables associated with a particular failure type and in the presence of competing risks. Multivariable models of cumulative incidence were fit using forward selection, with variables no longer entering the model if $P > .05$, unless deemed clinically important. All models were adjusted for monotherapy with ibrutinib vs combination therapy with ibrutinib and ofatumumab. Other variables considered included age; sex; number of prior therapies; baseline lactate dehydrogenase (LDH) level; fluorescence in situ hybridization (FISH) abnormalities del(17p), del(11q), trisomy 12, del(13q); *MYC* abnormalities; *BCL6* abnormalities; and complex karyotype and *IGHV* mutational status. Survival following discontinuation was calculated from the off-study date until the date of death from any cause, censoring patients at last contact. Survival estimates were calculated by the Kaplan-Meier method, and differences between curves were tested with the log-rank test. All tests were 2-sided, and statistical significance was declared at α=.05. The cmprsk package within TIBCO Spotfire S+ Version 8.2 was used to analyze time-to-event data in the presence of competing risks, whereas SAS statistical software Version 9.3 (SAS Institute Inc) was used for all other data analyses.

Results

Patient Characteristics and Rate of Ibrutinib Therapy Discontinuation

A total of 308 patients were included in this analysis, with data locked as of June 9, 2014. This included 237 patients treated with ibrutinib as a single agent in 3 clinical trials and 71 patients treated with ibrutinib in combination with ofatumumab. Clinical characteristics for all patients are given in **Table 1**. Three patients treated with ibrutinib plus ofatumumab had active Richter's transformation (RT) at study entry. Patients were generally high risk, with a median of 3 prior therapies (range, 0-16; 8 patients were previously untreated), 80% with unmutated *IGHV*, 58% with complex karyotype (3 or more distinct abnormalities), 37% with del(17p13.1), and 26% with del(11q22.3). With a median follow-up of 20 months (range, 2-47 months), 232 patients (75%) remain on therapy, including 7 patients who were removed from the study to pursue stem cell transplant or to receive ibrutinib therapy commercially at another center. Of the 76 patients who discontinued therapy, only 31 (10% of 308 total) discontinued therapy because of disease progression.

Nonrelapse-Related Discontinuation

Forty-five patients discontinued therapy for reasons other than disease progression. Twenty-eight discontinued because of infection, 8 because of other adverse events, and 9 owing to other events including progressive multifocal leukoencephalopathy in 1 patient who received rituximab 363 days prior, medication noncompliance (n = 1), comorbid medical condition (n = 2), failure to thrive (n = 1), sudden cardiac death (n = 1), need for anticoagulation (n=2), and cerebrovascular event (n = 1). Nonrelapse discontinuations tended to occur early, with only 1 patient discontinuing therapy after 24 months for a reason other than progressive disease. Therapy-limiting infections especially tended to occur early, with a median time to discontinuation of 102 days. Sixteen discontinuations due to infectious toxic effects occurred during the first 6 months of therapy, 7 within 6 to 12 months, and 5 beyond 12 months.

In multivariable modeling, age was the only significant independent risk factor of therapy discontinuation for reason other than disease progression (hazard ratio [HR] for 10-year increase, 1.87; 95% CI, 1.33-2.64 [$P < .001$]; **Table 2**). Increasing number of prior therapies was also associated with nonrelapse discontinuation, although it was not statistically significant (HR, 1.09; 95% CI, 1.00-1.19 [$P = .054$]).

Patients who discontinued ibrutinib therapy for nonrelapse reasons tended to do poorly, with a median survival after discontinuation of 8 days (95% CI, 0-56 days). This was heavily influenced by patients coming off trial because of infection, where 16 of the 28 patients with infection died the same day they came off treatment and only 2 were alive at the last fol-

Table 2. Multivariable Models for Cumulative Incidence of 2 Failure Types: Disease Progression and Toxicity, Adjusted for Monotherapy

	Event			
	Progression		Toxicity	
Variable	HR (95% CI)[a]	*P* Value	HR (95% CI)[a]	*P* Value
Age, 10-y increase	NA	NA	1.87 (1.33-2.64)	<.001
No. of prior treatments, 1 unit increase	NA	NA	1.09 (1.00-1.19)	.054
BCL6 abnormality, yes vs no	2.70 (1.25-5.85)	.01	NA	NA
Complex karyotype, yes vs no	4.47 (1.50-13.34)	.007	NA	NA

Abbreviations: HR, hazard ratio; NA, not applicable.

[a] An HR greater than 1 (lower than 1) indicates a higher (lower) risk of an event for the first category listed for dichotomous variables or increasing values of continuous variables.

low-up on days 63 and 176. Conversely, patients who discontinued therapy for noninfectious adverse events or other reasons had a median survival of 238 days, where 7 of the 17 patients had not died at the time of the last follow-up and 4 had survived more than a year following treatment discontinuation (eFigure 1 in the Supplement).

Discontinuation Due to Disease Progression

Of 31 patients who discontinued therapy because of disease progression, 13 progressed with CLL and 18 with RT, 3 of whom had previously transformed. Characteristics of these patients are listed in **Table 3**. While most of the patients with RT developed diffuse large B-cell lymphoma (78%), 1 patient each developed Hodgkin lymphoma, plasmablastic lymphoma, composite B-cell and T-cell lymphoma, and peripheral T-cell lymphoma. Richter's transformation tended to occur earlier than progressive disease because of CLL, where the estimated cumulative incidence of RT at 12 months was 4.5% (95% CI, 2.0%-7.0%) and the estimated cumulative incidence of progressive disease due to CLL at 12 months was 0.3% (95% CI, 0%-1.0%) (**Figure**).

Owing to the low frequency of relapse, failures due to RT or CLL were combined in an attempt to identify baseline factors that increased the risk of disease progression. In modeling individual variables associated with disease-related discontinuation and adjusting for monotherapy with ibrutinib, increased number of prior therapies (HR, 1.12; $P = .03$) *BCL6* abnormalities (HR, 3.77; $P < .001$), *MYC* abnormalities (HR, 2.59; $P = .01$), presence of del(17p) (HR, 2.28; $P = .03$), and complex karyotype (HR, 5.17; $P = .003$) were all significantly associated with a higher risk of progression (eTable 2 in the Supplement). In multivariable analysis, presence of *BCL6* abnormalities (HR, 2.70; 95% CI, 1.25-5.85 [$P = .01$]) and complex karyotype (HR, 4.47; 95% CI, 1.50-13.34 [$P = .007$]) remained independent risk factors (Table 2). Increased LDH level at baseline was not significantly associated with disease progression when combining failure types but appeared to be associated with development of RT.

Patients with RT generally did poorly following relapse. Of 18 patients, 6 were unable to receive further therapy and died within 1 month of transformation. One patient was treated at another institution with an unknown regimen. The remaining 11 patients were treated with R-EPOCH (rituximab plus etoposide, prednisolone, vincristine, cyclophosphamide, and doxorubicin) (n = 6), R-CHOP (rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone) (n = 1), R-ICE (rituximab plus ifosfamide, carboplatin, etoposide) (n = 1), rituximab plus dexamethasone (n = 1), OFAR (oxaliplatin, fludarabine, cytarabine, rituximab) (n = 1), or brentuximab (n = 1). One patient was lost to follow-up after treatment was started, but the 10 remaining patients all had disease progression during their next treatment regimen. Median survival from transformation was 3.5 months (95% CI, 10 days to 6.0 months) (eFigure 2 in the Supplement).

Patients with CLL progression tended to have rapid disease progression following discontinuation of ibrutinib therapy. One patient had rapid progression of nodes at another facility and was not seen again at OSU, so details of care are unknown. One patient had primarily progression in ascites and cytopenias and was therefore unmeasurable. Of the remaining 11 patients, 2 had disease progression in the peripheral blood only, 3 had progression in the nodes only, and 6 had both. For patients with peripheral blood disease (n = 8), the median increase in absolute lymphocyte count during the 6 months prior to ibrutinib therapy discontinuation was 17.34×10^9/L (range $6.62\text{-}177.34 \times 10^9$/L) (to convert to lymphocytes per microliter, divide by .001). All patients had a normal lymphocyte count 6 months prior to therapy discontinuation. For 5 patients where there was a time interval of more than 1 week between discontinuation of ibrutinib therapy and start of next therapy, the mean change in lymphocyte count between relapse and ibrutinib discontinuation was $+0.75 \times 10^9$/L per day, and was $+4.6 \times 10^9$/L per day from the time of ibrutinib therapy discontinuation until next therapy. For the patients with nodal disease (n = 9), the median increase in the sum of the products of lymph node diameter of the 3 largest nodes on the computed tomographic scan was 10.85 cm (range, 3.98-104.15 cm). Three patients had palpable lymph nodes and a time of more than 1 week between ibrutinib therapy discontinuation and next therapy. In these patients, the rate of change of the sum of the products of the 3 largest lymph nodes by physical examination was +0.35 cm, 0 cm, and 0.2 cm per day, respectively, prior to ibrutinib therapy discontinuation, and +0.79 cm, 1.18 cm, and 4.63 cm per day after ibrutinib therapy discontinuation.

After disease progression, patients tended to require the initiation of therapy quickly to achieve disease control (Table 3). Of the 13 patients, 11 received further therapy, most within a few weeks of discontinuing ibrutinib therapy (median, 10 days; range, 1-42 days). Two were not able to receive further therapy and died at days 10 and 180, respectively, following discontinuation. Median survival from time of CLL progression was 17.6 months (95% CI, 4.7 months–"not reached") (eFigure 2 in the Supplement).

Table 3. Characteristics of Patients Who Experienced Relapse During Ibrutinib Therapy

Type of Relapse	Monotherapy vs Combination With Ofatumumab	Baseline FISH Findings [Complexity of Karyotype]	Days on Study	Primary Site of Relapse	Time From Ibrutinib Therapy Discontinuation Until Next Therapy
DLBCL	Monotherapy	Del(17p), Del(13q), *MYC* [complex]	26	LN	18
DLBCL	Monotherapy	Del(17p), Del(11q), *MYC*, *BCL6* [complex]	55	LN	21
DLBCL	Monotherapy	Del(13q), *MYC* [complex]	125	LN	21
DLBCL	Monotherapy	Del(17p), +12 [complex]	170	LN	No treatment
DLBCL	Complex	[Complex]	231	LN	7
DLBCL	Monotherapy	Del(17p), +12, *MYC* [complex]	261	LN	No treatment
DLBCL	Monotherapy	Del(11q), *MYC* [complex]	271	LN	1
DLBCL	Monotherapy	Del(17p), Del(11q), +12, *MYC*, *BCL6* [complex]	309	LN	1
DLBCL	Monotherapy	Unknown	387	LN	24
DLBCL	Combination	Del(17p) [complex]	429	LN, peripheral blood (CLL)	No treatment
DLBCL	Monotherapy	Del(17p), Del(13q) [complex]	562	LN	No treatment
DLBCL	Monotherapy	del(17p), *MYC*, *BCL6* [complex]	785	LN, peripheral blood (CLL)	12
DLBCL progression[a]	Combination	Del(13q), *MYC* [complex]	479	LN	11
DLBCL progression[a]	Combination	*BCL6* [complex]	168	LN	33
Composite B- and T-cell lymphoma	Combination	+12, Del(13q) [not complex]	377	LN	No treatment
Hodgkin lymphoma	Monotherapy	Del(17p), Del(11q), Del(13q) [complex]	308	LN	44
Plasmablastic lymphoma	Monotherapy	Del(11q), Del(13q) [not complex]	337	LN	21
Peripheral T-cell lymphoma	Monotherapy	Normal FISH [unknown]	33	LN	No treatment
CLL	Monotherapy	Del(13q) [complex]	474	LN, peripheral blood	1
CLL	Monotherapy	Normal FISH [complex]	511	LN, peripheral blood	12
CLL	Monotherapy[b]	Del(11q), *BCL6* [complex]	664	Peripheral blood	21
CLL	Monotherapy	del(17p), *MYC* [complex]	965	LN, peripheral blood	8
CLL	Monotherapy	Del(11q) [not complex]	1119	LN, peripheral blood	2
CLL	Monotherapy	Del(17p), Del(13q), *MYC*, *BCL6* [complex]	1216	LN, peripheral blood	29
CLL	Monotherapy	Del(17p) [complex]	1295	LN only	1
CLL	Combination	Del(11q), Del(13q) [not complex]	115	Ascites	16
CLL	Combination	Del(17p) [complex]	426	Unknown	Unknown
CLL	Combination[b]	Del(17p) [complex]	505	Peripheral blood	2
CLL	Combination[b]	Normal FISH [complex]	673	LN only	42
CLL	Combination	Del(17p), Del(13q), *MYC* [complex]	693	LN, peripheral blood	17
CLL	Combination	Del(17p), Del(13q), *MYC*, *BCL6* [complex]	1034	LN only	1

Abbreviations: CLL, chronic lymphocytic leukemia: DLBCL, diffuse large B-cell lymphoma; FISH, fluorescence in situ hybridization; LN, lymph node.

[a] Patients with history of Richter's transformation at study entry.

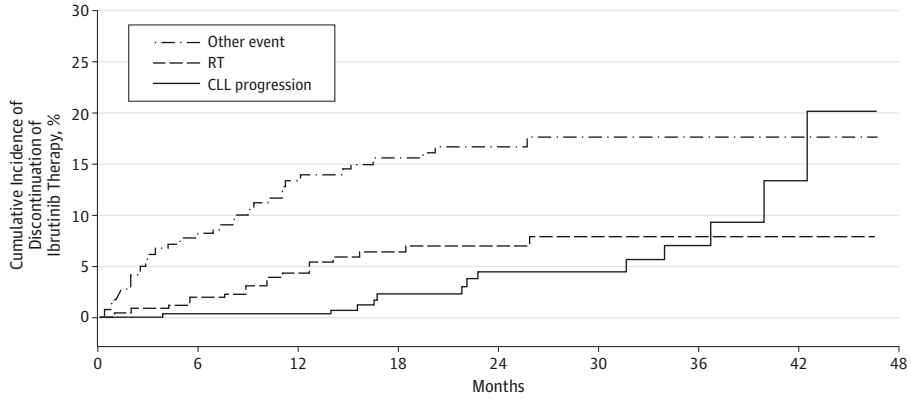
[b] Patients previously reported.[19]

Association of *BTK* and *PLCG2* Mutations With Relapse During Ibrutinib Therapy

We have previously shown that resistance to ibrutinib can be mediated through mutations in *BTK* or *PLCG2* in a small group of patients. To extend these findings, we have performed Ion Torrent deep sequencing for both of these genes at baseline and time of relapse for patients who have relapsed on ibrutinib therapy (**Table 4**).

In patients who progressed with RT, peripheral blood was obtained at baseline and time of relapse in 8, whereas lymph node biopsy was obtained at relapse from 1 patient. The lymph node at progression showed no mutations in *BTK* or *PLCG2*. Peripheral blood in 6 patients at disease progression was negative for mutations, but 2 patients had mutations: 1 had 3 separate mutations in *BTK* (C481S, T474I, and T474S), and 1 had 3 separate mutations in *BTK* (C481Y, C481R, and L528W) and

Figure. Cumulative Incidence of Discontinuation of Ibrutinib Therapy



No. at Risk: 308 261 169 135 86 58 34 10 0

Cumulative Incidence Estimates, % (95% CI)

	At 12 mo	At 18 mo
CLL progression	0.3 (0-1.0)	2.4 (0.3-4.6)
RT	4.5 (2.0-7.0)	6.5 (3.3-9.6)
Other event	13.5 (9.5-17.6)	15.6 (11.1-20.0)

Rate of discontinuation is low overall, with relapse-related discontinuations less frequent than nonrelapse related discontinuations. Richter's transformation (RT) tends to occur earlier than progression with typical chronic lymphocytic leukemia (CCL).

Table 4. Ion Torrent Deep Sequencing of 13 Patients Who Have Relapsed on Ibrutinib in Which Mutations Have Been Identified in *BTK* or *PLCG2* [a]

Patient No.	Type of Relapse	Chromosome	Gene	AA Change	Baseline		Relapse	
					Coverage	Variant Frequency, %	Coverage	Variant Frequency, %
1	CLL	16	*PLCG2*	R665W	870	0	9977	5.5
		16	*PLCG2*	S707P	2940	0	9980	5.3
		16	*PLCG2*	S707F	2937	0	9996	3.3
		16	*PLCG2*	R742P	1623	0	9987	6.1
		16	*PLCG2*	L845fs	1200	0	5436	14.9
2	CLL	16	*PLCG2*	R665W	3230	0	9252	45.0
3	CLL	X	*BTK*	C481F	1837	0	6687	84.0
4	CLL	X	*BTK*	C481S	2730	0	4417	16.9
5	CLL	16	*PLCG2*	R665W	3465	0	2781	2.0
		16	*PLCG2*	S707Y	8456	0	2346	6.6
		16	*PLCG2*	L845F	3431	0	1025	23.8
		X	*BTK*	C481S	875	0	2429	6.8
6	CLL	X	*BTK*	C481S	2086	0	5903	37.8
7	CLL	X	*BTK*	C481S	4453	0	490	47.5
8	CLL	X	*BTK*	C481S	1210	0	2689	74.7
9	CLL	X	*BTK*	C481S	2260	0	8699	6.0
10	CLL	16	*PLCG2*	D1140G	1372	0	5974	13.5
		X	*BTK*	C481S	3109	0	6941	13.0
11	CLL	X	*BTK*	C481S	1119	0	4638	5.4/3.4[b]
12	RT	X	*BTK*	C481S	1622	0	497	51.9
		X	*BTK*	T474I	264	0	112	4.0
		X	*BTK*	T474S	264	0	112	4.0
13	RT	16	*PLCG2*	D334H	629	0	27622	5.7
		X	*BTK*	C481Y	487	0	16800	28.7
		X	*BTK*	C481R	488	0	17699	24.2
		X	*BTK*	L528W	310	0	9994	7.9

Abbreviations: CLL, chronic lymphocytic leukemia; RT, Richter's transformation.

[a] Seven patients with RT did not have mutations identified in *BTK* or *PLCG2* with similarly deep coverage and are not included.

[b] Patient with 2 different mutations that both resulted in C481S change.

D334H mutation in *PLCG2*. Notably, these 2 patients were the only patients with RT who had a worsening lymphocytosis with CLLphenotype at the same time as RT, so it is possible that they had both progressive CLL as well as RT.

In patients who had CLL progression, samples at baseline (peripheral blood) and at the time of relapse was obtained in 11 of 13 patients (peripheral blood in 9 and bone marrow in 2). Three of these patients have been previously reported[19] and had acquired mutations in *BTK* C481S, *PLCG2* R665W, and *BTK* C481S in association with *PLCG2* R665W, L845F, and S707Y. In the 8 remaining patients, 5 had sole mutations in *BTK* with C481S, 1 had *BTK* C481F mutation, and 1 had 5 separate mutations in *PLCG2* (R665W, S707P, S707F, R742P, and L845fs). One additional patient had both *BTK* and *PLCG2* mutations, with *BTK* C481S and *PLCG2* D1140G. Thus, all patients who have been identified at our institution to have progressive CLL after ibrutinib therapy have had mutations in either *BTK* or *PLCG2* that were acquired during therapy. Relapse mutations were not identified before treatment in any patient with deep sequencing (Table 4).

Discussion

We describe herein the first large single-center experience of patients treated with ibrutinib, with a median follow-up of almost 2 years. We show that nonrelapse-related discontinuation of therapy is more common early in therapy and becomes progressively infrequent. We show that RT and CLL progressions are both relatively uncommon in this very high-risk population, with RT tending to occur earlier and CLL progression later. Finally, we confirm and extend our previous findings that CLL progression is associated with mutations in *BTK* or *PLCG2*.

While previous publications of clinical trials report reasons for discontinuation of ibrutinib therapy, this report greatly extends these findings by including a larger number of patients than any individual trial and longer follow-up than other reports. Also, we report for the first time to our knowledge a multivariable analysis identifying factors at baseline that are associated with discontinuation and outcomes following ibrutinib therapy discontinuation, which will be relevant for general oncology practice.

Perhaps most significant, these data confirm that ibrutinib is a drug capable of shifting the paradigm of therapy for relapsed disease. In our group of high-risk patients, the estimated cumulative incidence of disease progression at 18 months was only 8.9% (95% CI, 5.2%-12.6%). As more patients are treated with ibrutinib off study, we expect that outcomes will not be as positive as in clinical trials, given variability in compliance and follow-up, but we should see patients with relapsed CLL living longer than has been seen in the past. As our data show an estimated cumulative incidence of nonrelapse discontinuation of 15.6% (95% CI, 11.1%-20.0%) at 18 months, there remains a need to identify new therapies for patients who do not tolerate ibrutinib or who are unable to continue because of concomitant medications.

These data also confirm and extend earlier reports of the association of *BTK* and *PLCG2* mutations with CLL relapse. Interestingly, in our experience thus far, these mutations are associated with all CLL relapses. While we have not yet characterized all of these mutations, our research thus far has shown that *BTK* C481 mutations interfere with ibrutinib binding, and *PLCG2* mutations potentially have gain-of-function effects. We are currently characterizing the additional mutations identified as part of this analysis. From this small subset of patients, it appears that these mutations may help to identify a patient who is relapsing with CLL. Importantly, we still do not see evidence of these mutations at baseline, suggesting that they are indeed the result of selective pressure from the drug, although sampling only peripheral blood compartment is a limiting factor, and it is possible that these mutations may be present in the blood at very low levels or in other niches at baseline. Finally, our data suggest that RT is not associated with these mutations in most cases and opens the question of resistance mechanisms in this patient population.

While this analysis presents data from a large number of patients and we attempt to control for confounding variables through multivariable analyses, our study is limited by the retrospective nature of the data and the single-center experience. It is possible that patterns at our institution may not be generalizable to the entire ibrutinib-treated population, and it will be important to confirm these findings in larger multicenter patient populations. However, given that this drug is relatively new and discontinuations are infrequent with short follow-up, it will likely be many years before larger series with longer follow-up times are available.

Ultimately, we show that patients with disease progression while on ibrutinib therapy, especially those with RT, have poor outcomes. While the incidence of disease progression is low with this duration of follow-up, it is expected that more patients will experience relapse with extended follow-up. We show that disease tends to progress quickly in patients, especially when the drug therapy is stopped, which points to a need for clinical trials to allow shorter washout periods for these patients. Also, physicians may consider waiting before discontinuing ibrutinib therapy until an alternative therapeutic plan can be formed and potentially even continuing ibrutinib therapy in combination with RT-directed therapy in select patients.

Conclusions

These data show that continued clinical trials are needed in this disease and that scientific progress cannot stop even with a breakthrough drug like ibrutinib. Patients with RT remain a high research priority to identify new targets and new therapies. Also, the question of whether high-risk patients may benefit more from transplant or kinase inhibitor is unanswered. There remains much to be learned about the biological mechanisms of CLL from the use of kinase inhibitor drugs, and the ideal drug, target, or combination for all patients is likely yet to be identified.

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