REGULAR ARTICLE

blood advances

Near-tetraploidy is associated with Richter transformation in chronic lymphocytic leukemia patients receiving ibrutinib

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Key Points

- Pretreatment near-tetraploidy is associated with advanced Rai stage, deletion of 17p, and complex karyotype.
- Pretreatment near-tetraploidy is an independent risk factor for ibrutinib discontinuation via Richter transformation.

Ibrutinib is a highly effective targeted therapy for chronic lymphocytic leukemia (CLL). However, ibrutinib must be discontinued in a subset of patients due to progressive CLL or transformation to aggressive lymphoma (Richter transformation). Transformation occurs early in the course of therapy and has an extremely poor prognosis. Thus, identification of prognostic markers associated with transformation is of utmost importance. Near-tetraploidy (4 copies of most chromosomes within a cell) has been reported in various lymphomas, but its incidence and significance in CLL has not been described. Using fluorescence in situ hybridization, we detected near-tetraploidy in 9 of 297 patients with CLL prior to beginning ibrutinib treatment on 1 of 4 clinical trials (3.0%; 95% confidence interval [CI], 1.4%-5.7%). Near-tetraploidy was associated with aggressive disease characteristics: Rai stage 3/4 ($P = .03$), deletion 17p ($P = .03$), and complex karyotype ($P = .01$). Near-tetraploidy was also associated with ibrutinib discontinuation due to Richter transformation ($P < .0001$), but not due to progressive CLL ($P = .41$). Of the 9 patients with near-tetraploidy, 6 had Richter transformation with diffuse large B-cell lymphoma. In a multivariable model, near-tetraploidy (hazard ratio [HR], 8.66; 95% CI, 3.83-19.59; $P < .0001$) and complex karyotype (HR, 4.77; 95% CI, 1.42-15.94; $P = .01$) were independent risk factors for discontinuing ibrutinib due to transformation. Our results suggest that near-tetraploidy is a potential prognostic marker for Richter transformation to assess in patients going on ibrutinib.

Introduction

Ibrutinib is a first-in-class oral covalent inhibitor of Bruton tyrosine kinase (BTK)[1] approved to treat chronic lymphocytic leukemia (CLL), mantle cell lymphoma, and Waldenstrom macroglobulinemia. Ibrutinib has rapidly changed the landscape of CLL treatment, with high response rates and prolonged remission durations in both relapsed/refractory CLL and previously untreated patients.[2-5] Despite these strides, a subset of patients relapse on ibrutinib. Patients relapse primarily with progressive CLL or Richter transformation, an aggressive transformation into lymphoma, predominantly diffuse large B-cell lymphoma.[6] Most patients progressing with CLL acquire mutations in either the C481 ibrutinib-binding pocket of *BTK* or downstream activating mutations in *PLCG2* that bypass the need for signaling through BTK.[7,8] However, identifiable resistance mutations are considerably less frequent in patients who

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Table 1. Clinical and molecular characteristics of near-tetraploid patients

Characteristics	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
Classification for discontinuation	CLL	Richter	Richter	Richter	Richter	Richter	Richter	–	–
Study	p12024	p10032	p10032	p11133	p10053	p12024	p11133	p10053	p10053
Ibrutinib monotherapy	Y	Y	Y	Y	N	Y	Y	N	N
Age, y	60	76	60	69	73	53	52	79	64
Sex	M	M	M	M	M	F	F	F	F
Rai stage	IV	IV	III	IV	IV	IV	IV	IV	IV
LDH, U/L	214	375	142	211	765	308	198	356	227
Elevated LDH	Y	Y	N	Y	Y	Y	Y	Y	Y
No. of prior therapies	4	5	5	3	5	3	8	5	2
Gain of *BCL6*	Y	N	N	N	Y	N	N	N	N
Gain of *MYC*	Y	N	N	Y	N	Y	Y	N	N
Trisomy 12	Y	N	N	Y	N	Y	N	Y	N
del(13q)	Y	N	Y	N	N	N	N	Y	Y
del(11q)	N	N	Y	N	N	N	N	Y	N
del(17p)	Y	Y	N	Y	N	Y	Y	Y	Y
Complex karyotype	Y	Y	Y	Y	Y	Y	Y	Y	Y
IGHV	UM	UM	UM	UM	Unknown	Unknown	UM	UM	UM
Time to discontinuation or last contact, y	2.50	2.15	1.06	0.85	0.46	0.71	2.20	5.38	4.33
Richter treatment	–	R + DXM	OFAR	R-EPOCH	R-EPOCH	Unknown	Unknown	–	–
OS, y	3.77	2.48	2.08	1.01	0.83	0.73	3.97	5.38	4.33
Survival status	A	D	D	D	D	D	A	A	A

–, Not applicable; A, alive; D, dead; del, deletion; F, female; LDH, lactate dehydrogenase; M, male; N, no; OFAR, oxaliplatin, fludarabine, cytarabine, and rituximab; R + DXM, rituximab and dexamethasone; R-EPOCH, rituximab, etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin; UM, unmutated; Y, yes.

progress via disease transformation.[6] Identifying prognostic markers associated with Richter transformation is critical at this time as an increasing number of patients have begun to receive ibrutinib and those whose CLL transforms have very aggressive disease and poor prognosis.[6,9] Complex karyotype (≥3 unrelated chromosomal abnormalities) has been associated with ibrutinib discontinuation due to progressive CLL or transformation.[6,10] In addition, various biomarkers in CLL have been associated with risk of Richter transformation, including inactivation of TP53 or CDKN2A, *CMYC* abnormalities, *NOTCH1* mutation, and CD38 expression.[11-16] However, for patients receiving ibrutinib, there are currently no specific risk factors associated with developing Richter transformation.

Near-tetraploidy has been reported as a rare abnormality in various lymphomas, but to our knowledge, its incidence in CLL has not been examined.[17,18] Tetraploidy in cancer cells may promote chromosome instability while buffering the cells from the deleterious effects.[19] In this study, we examined the presence of near-tetraploidy and its association with discontinuation of ibrutinib for progression, including Richter transformation.

Methods

All patients provided written informed consent to participate in protocols approved by The Ohio State University Cancer Institutional Review Board in accordance with the Declaration of Helsinki. Patient population characteristics and the 4 ibrutinib clinical trials have been described previously.[2,3,6,20,21] We retrospectively reviewed fluorescence in situ hybridization (FISH) results to identify near-tetraploid cases. Cytogenetic procedures were performed as previously described[22] on peripheral blood or bone marrow collected prior to starting ibrutinib therapy. For each patient, 7 to 10 probes, plus controls, were analyzed (supplemental Table 1). Given our probe set, we defined near-tetraploidy as the presence of 4 or more probes with 4 signals and confirmed each positive case by the presence of at least 1 near-tetraploid cell by karyotype.

Cytogenetic results were available for 297 patients. Baseline variables were compared between patients with and without near-tetraploidy using the Fisher exact test or the Wilcoxon rank-sum test. Time to treatment discontinuation was measured from the date of first treatment until the off-study date. We censored 134 patients who remained on ibrutinib at the date of last contact, and 13 patients who went off study for transplant or went offsite. Cumulative incidence of ibrutinib discontinuation due to progression or histologic transformation was estimated, with discontinuation for other reasons treated as a competing risk. The Gray test was used to test for cumulative incidence differences between groups. Multivariable models were fit using Fine and Gray models.[23] Variables considered for model inclusion were age, sex, number of prior therapies, baseline lactate dehydrogenase level, FISH abnormalities [del(17)(p13.1), del(11)(q22.3), trisomy 12, del(13)(q14), *MYC* gain, and *BCL6* gain], complex karyotype, and *IGHV* mutational status. The multivariable model for transformation adjusted for monotherapy with ibrutinib vs combination therapy with ibrutinib and ofatumumab, regardless of statistical significance. Overall survival (OS) was measured from the date of first treatment with ibrutinib until the date

Table 2. Near-tetraploidy is associated with aggressive disease characteristics

Characteristic	Total, n = 297	Not tetraploid, n = 288	Tetraploid, n = 9	P
Study, no. (%)				.25
OSU-10032	48 (16)	46 (16)	2 (22)	
OSU-10053	68 (23)	65 (23)	3 (33)	
OSU-11133	145 (49)	143 (50)	2 (22)	
OSU-12024	36 (12)	34 (12)	2 (22)	
Monotherapy with ibrutinib, no. (%)				.43
No	68 (23)	66 (23)	3 (33)	
Yes	229 (77)	223 (77)	6 (67)	
Age, y				.85
Median	65	65	64	
Range	26-91	26-91	52-79	
Sex, no. (%)				.29
Male	211 (71)	206 (72)	5 (56)	
Female	86 (29)	82 (28)	4 (44)	
Rai stage, no. (%)				.03
0/I/II	101 (34)	101 (35)	0 (0)	
III/IV	196 (66)	187 (65)	9 (100)	
LDH, U/L				.19
Median	220	220	227	
Range	96-1485	96-1485	142-765	
No. of prior therapies				.12
Median	3	3	5	
Range	0-16	0-16	2-8	
Gain of *BCL6*, no. (%)				.15
No	274 (92)	267 (93)	7 (78)	
Yes	23 (8)	21 (7)	2 (22)	
Gain of *CMYC*, no. (%)				.09
No	235 (79)	230 (80)	5 (56)	
Yes	62 (21)	58 (20)	4 (44)	
Trisomy 12, no. (%)				.05
No	245 (82)	240 (83)	5 (56)	
Yes	52 (18)	48 (17)	4 (44)	
Del(13q), no. (%)				.75
No	145 (49)	140 (49)	5 (56)	
Yes	152 (51)	148 (51)	4 (44)	
Del(11q), no. (%)				1.00
No	214 (72)	207 (72)	7 (78)	
Yes	83 (28)	81 (28)	2 (22)	
Del(17p), no. (%)				.03
No	177 (60)	175 (61)	2 (22)	
Yes	120 (40)	113 (39)	7 (78)	
Complex karyotype, no. (%)				.01
No	119 (41)	119 (42)	0 (0)	
Yes	172 (59)	163 (58)	9 (100)	
***IGHV*, no. (%)**				.35
Mutated	53 (20)	53 (21)	0 (0)	
Unmutated	212 (80)	205 (79)	7 (100)	

Associations between near-tetraploidy and demographic, clinical, and molecular variables. Associations were tested using the Fisher exact for categorical variables and Wilcoxon rank-sum tests for continuous variables.

of death, censoring those alive at last follow-up. OS estimates were calculated by the Kaplan-Meier method and differences between curves were tested with the log-rank test. All tests were 2-sided; statistical significance was declared at $\alpha = 0.05$.

Results

A near-tetraploid clone (supplemental Tables 2-4) was identified in 9 of 297 patients analyzed (3.0%; 95% confidence interval [CI], 1.4%-5.7%). Clinical and molecular characteristics for each of the 9 patients are described in Table 1. Near-tetraploidy was associated with the baseline characteristics Rai stage III/IV ($P = .03$), deletion 17p13 ($P = .03$), and complex karyotype ($P = .01$) (Table 2). With a median follow-up of 3.4 years, 133 patients (45%) remain on therapy, 12 (4%) received transplant or therapy elsewhere, 52 (18%) discontinued ibrutinib due to progressive CLL, 28 (9%) discontinued due to disease transformation, and 72 (24%) discontinued for other adverse events (supplemental Table 5). Among the 9 near-tetraploid patients, 6 have undergone Richter transformation, 1 had progressive CLL, and only 2 remain on treatment at 4.3 and 5.4 years. The estimated cumulative incidence of disease transformation is significantly higher in near-tetraploid patients vs those without near-tetraploidy ($P < .0001$; Figure 1A; supplemental Table 5), with an estimated cumulative incidence of disease transformation at 3 years of 66.7% (95% CI, 23.5-89.3) and 7.6% (95% CI, 4.8-11.1), respectively. Because all 9 near-tetraploid patients had a complex karyotype and complex karyotype has been associated with progression on ibrutinib,[6] cumulative incidence curves are presented for near-tetraploidy and complex karyotype (Figure 1B). Patients with neither abnormality had a low incidence of ibrutinib discontinuation due to transformation. Patients with only complex karyotype had a significantly higher cumulative incidence compared with patients without either feature ($P = .007$). Patients with both features had a significantly higher cumulative incidence compared with those with only complex karyotype ($P < .0001$). The importance of these variables was supported by a multivariable model in which both near-tetraploidy (hazard ratio = 8.66; 95% CI, 3.83-19.59; $P < .0001$) and complex karyotype (hazard ratio = 4.77; 95% CI, 1.42-15.94; $P = .01$) were independent risk factors for discontinuing ibrutinib due to transformation. Near-tetraploidy was not significantly associated with discontinuation due to progressive CLL ($P = .41$; supplemental Table 5). Near-tetraploidy showed a trend toward decreased OS ($P = .08$; Figure 1C; supplemental Table 5). Although the survival curve is lowest in patients who have both near-tetraploidy and complex karyotype and highest in patients with neither feature, the data were insufficient to claim that near-tetraploidy provided prognostic information for OS independent of complex karyotype

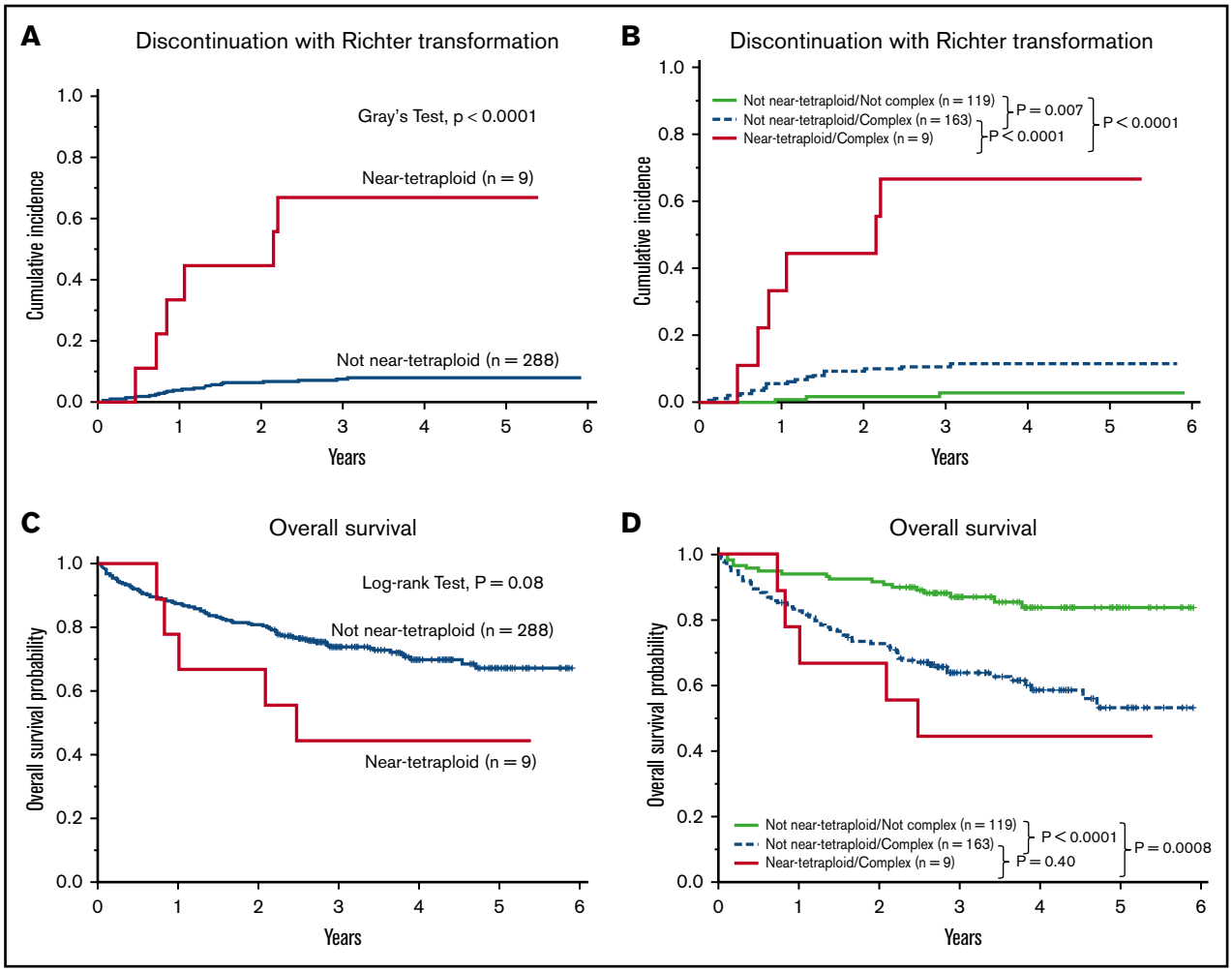

Figure 1. Near-tetraploidy is associated with Richter transformation on ibrutinib. (A) Cumulative incidence curves for transformation on ibrutinib with and without near-tetraploidy. (B) Cumulative incidence curves for transformation by near-tetraploidy and complex karyotype status. (C) Kaplan-Meier curves of OS for patients with or without near-tetraploidy. (D) Kaplan-Meier curves of OS by near-tetraploidy and complex karyotype status.

(P = .40; Figure 1D). The small number of patients with near-tetraploidy and complex karyotype, as well as the presence of 18 patients whose CLL had transformed, and the majority of CLL progressions being in the complex-karyotype-alone subgroup likely contributed to this nonsignificant finding.

Discussion

As a retrospective study, there are several limitations. The sampling time varied from days to months before beginning treatment. Because serial samples were unavailable for most patients, we were unable to determine whether the frequency of the near-tetraploid clones changed during treatment, or if gain of near-tetraploidy after starting treatment is associated with Richter transformation. We were also unable to assess whether the transformed lymphoma was clonally related to the preceding CLL. To address these questions, it would be useful to monitor patients serially for near-tetraploidy before and throughout treatment, as well as analyze lymph node tissue at the time of transformation. Interestingly, in 3 near-tetraploid patients with FISH results available near the time of transformation, the near-tetraploid clone was still present, whereas for the 2 patients who have not progressed, the near-tetraploid clone is no longer detectable.

Our study suggests that near-tetraploidy may be a prognostic marker for progression on ibrutinib through Richter transformation and provides rationale for further interrogation of this biomarker in CLL. It will be important to confirm the relationship with Richter transformation on ibrutinib in a second, independent cohort, as well as to examine the frequency and clinical associations of near-tetraploidy with transformation in other settings (eg, other therapies, at diagnosis, etc). Using FISH probes for regions prone to variation in CLL complicates the identification and quantification of near-tetraploidy, thus there is a need for further research to optimize detection. Near-tetraploid cells are typically unstable and this may serve as a mechanism for generating diverse subclones that could promote transformation in CLL. Future studies to interrogate downstream effects and identify the mechanisms driving near-tetraploidy in CLL are of considerable interest.

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Authorship

Contribution: C.R.M., A.S.R., J.A.W., and J.C.B. wrote the manuscript; C.R.M., A.S.R., N.A.H., K.J.M., J.L., H.B., G.L., J.S.B., K.A.R., F.T.A., W.Z., A.L.G., J.A.J., J.M.F., S.M.J., L.A.A., K.A.B., M.R.G., A.J.J., L.V.A., E.K.H., J.A.W., and J.C.B. collected and analyzed data, reviewed the paper, and approved the final version; and J.A.W. and J.C.B. supervised the study.

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