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Variations of the ataxia telangiectasia mutated gene in patients with chronic lymphocytic leukemia lack substantial impact on progression-free survival and overall survival: a Cancer and Leukemia Group B study

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Abstract

The impact of mutation of the ATM (ataxia telangiectasia mutated) gene in chronic lymphocytic leukemia (CLL) treatment outcome has not been examined. We studied ATM mutations in 73 patients treated with fludarabine and rituximab. ATM gene mutation analysis was performed using temperature gradient capillary electrophoresis. The impact of detected variants on overall survival (OS) and progression-free survival (PFS) was tested with proportional hazards models. None of the 73 patients demonstrated truncating ATM mutations; 17 (23%, 95% confidence interval 14 – 35%) had non-silent variants (ATM-NSVs), including 13 known ATM polymorphisms and four missense variants. ATM-NSVs were not significantly associated with any baseline characteristics including immunoglobulin heavy chain variable gene (IGVH) status. In multivariable models, no significant differences in complete response ($p = 0.70$), PFS ($p = 0.59$) or OS ($p = 0.13$) were observed. Our data indicate that truncating ATM mutations are rare in patients with CLL. Furthermore, in this dataset, these non-silent variants had limited impact on PFS and OS.

Keywords

Chronic lymphocytic leukemia; ATM mutation; prognosis; chemoimmunotherapy

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Supplementary material available online Details of PCR primers, conditions, TGCE method and databases used

Introduction

Chronic lymphocytic leukemia (CLL) is the most common type of adult leukemia. Due to lack of survival advantage of early therapy in asymptomatic patients, CLL is observed without treatment until symptoms develop. An unmutated immunoglobulin heavy chain variable gene (IGVH) status [1 – 3] and deletions of 17p13.1 and 11q22.3 [4 – 8] are predictors of rapid time to progression (requiring therapy), short overall survival and poor response to therapy in treated patients [4,5]. These parameters are currently accepted and used for predicting the clinical outcome of newly diagnosed patients with CLL. However, as specified by the International Workshop on Chronic Lymphocytic Leukemia (IWCLL) 2008 criteria, they are not used to justify early treatment of symptomatic patients outside of clinical trials [9]. Similarly, the IWCLL response criteria guidelines recommend that, at present, only del(17p13.1) abnormality or p53 mutation definitively warrants treatment different from that of other patients outside of clinical trials [9].

First-line chemotherapy for CLL includes alkylators (chlorambucil, cyclophosphamide) and nucleoside analogs (fludarabine, cladribine, pentostatin), administered either as monotherapy or combined [10,11]. Rituximab has recently been added to chemotherapy based upon improved treatment outcome and overall survival with this drug in combined chemoimmunotherapy [12]. Both alkylators and nucleoside analogs kill CLL cells through the induction of DNA damage, and require intact cellular pathways that respond to DNA damage, including p53 and ATM (ataxia telangiectasia mutated gene). The strong negative impact of 17p13.1 and 11q22.3 deletion in CLL is presumed to be secondary to loss of p53 and ATM genes, respectively [4 – 8,13]. Inactivation of the p53 gene is further corroborated by several studies that demonstrate a negative impact of p53 gene mutation on CLL outcome regardless of the deletion status of 17p13.1 [14 – 19]. Similarly, patients with del(11q22.3) have an inferior outcome with fludarabine [20] or fludarabine – rituximab [4] containing therapy, but appear to have an improved outcome with fludarabine – cyclophosphamide (FC) [11] or FC with rituximab [21]. Mutation of the ATM gene in CLL has been well described [22 – 29]. However, data addressing the impact of ATM gene mutation in CLL relative to disease progression and its impact on treatment, especially in the absence of 11q22.3 deletion, are limited [30 – 32].

To better understand the impact of ATM gene mutation on the outcome of patients with CLL treated with chemoimmunotherapy, we studied the influence of ATM mutation on progression-free survival (PFS) and overall survival (OS) using pretreatment samples derived from Cancer and Leukemia Group B (CALGB) 9712 study patients [33]. Herein we provide evidence that (1) truncating mutations of ATM were absent in a cohort of sporadic therapy-naive patients with CLL in the USA, and (2) non-silent missense variants of ATM (ATM-NSVs) had minimal impact on both PFS and OS following the receipt of fludarabine and rituximab-based chemoimmunotherapy.

Design and methods

Subjects

Patients were enrolled on CALGB 9712 [33] and the corresponding tissue bank study (CALGB 9665) following written informed consent. Eligibility criteria included symptomatic, but untreated, immunophenotypically and histologically documented CLL, defined by the National Cancer Institute 1996 (NCI 96) guidelines [34]. The study was designed to compare the effect of rituximab administered concurrently ($n = 51$) or sequentially ($n = 53$) with fludarabine therapy. A total of 73 patients (70%) had a cryopreserved specimen available for analysis.

ATM mutation screening

Samples—Cryopreserved CLL cells isolated from blood specimens collected prior to the initiation of treatment were obtained from the CALGB Leukemia Tissue Bank at Ohio State University (OSU). DNA was extracted using a Qiagen QIAmp DNA Minikit (Qiagen Inc., Valencia, CA) according to the manufacturer's instructions.

ATM PCR conditions and TGCE analysis—All coding exons of *ATM* (4 – 65) were analyzed using polymerase chain reaction (PCR) amplification with primers designed to cover the entire exon and adjacent fragments of introns, including splicing donor and acceptor sites. Specimens were heteroduplexed with exon matched control DNA, and screened using temperature gradient capillary electrophoresis (TGCE) with the Reveal System (Spectru- Medix/Transgenomic). Samples with abnormal peaks were purified using a Qiagen QiaQuick PCR purification kit (Qiagen Inc.) according to the manufacturer's protocol. Twenty nanograms of purified PCR product were sent to the OSU Nucleic Acid Shared Resources laboratory for bidirectional sequencing. The sequence was compared with the ATM reference sequence using LaserGene6 Software (DNA Star Inc., Madison, WI). Sequence changes were described according to Human Genome Variation Society criteria and categorized using several databases. Details of PCR primers, conditions, the TGCE method and databases used are provided in the Supplementary Appendix (Supplementary Tables 1A – 1C).

Clinical endpoints—Responses were defined by NCI 96 criteria [34]. PFS was calculated from the on-study date to date of progression or death, whichever came first, censoring patients alive and progression-free at last follow-up. OS was calculated from the on-study date to date of death or date last alive.

Statistical methods—Associations between patients with and without ATM – NSVs and baseline features were analyzed using Fisher's exact or Wilcoxon rank-sum test. OS and PFS estimates were calculated using the Kaplan – Meier method, and the log-rank test evaluated differences between survival distributions. Proportional hazards and logistic regression models were fit using a limited backward-selection procedure to determine whether the presence of ATM – NSVs was associated with PFS/OS and complete response, respectively, independent of other prognostic factors. Variables considered for the multivariable model included age, sex, Rai stage (high vs. low), log-transformed leukocyte count (white blood cells; WBC) and lactate dehydrogenase (LDH), as well as IGVH status and presence of poor cytogenetics [del(17p13.1)/ del(11q22.3) vs. other]. The high-risk genomic groups were combined based upon similar outcome [4] and also the small number of patients with del(17p13.1) ($n = 3$) in this study. Variables, other than ATM-NSV, with the largest p -values were sequentially removed until the only variables remaining in the model were significant at $\alpha = 0.05$. Follow-up data on all patients were locked in October 2011. All analyses were performed by the Alliance for Clinical Trials in Oncology Statistics and Data Center.

Results

Associations of ATM variant status with clinical characteristics and outcome

Baseline characteristics of the 73 patients studied are summarized in Table I. None of the patients showed truncating ATM mutations; ATM-NSVs were detected in 17 patients (23%, 95% confidence interval [CI] 14 – 35%). One patient had two ATM-SNVs whereas the remainder had one. There were no significant differences in baseline characteristics between patients with and without ATM-NSVs (Table I), including IGVH mutational status ($p = 1.00$). Treatment outcome as measured by response, PFS and OS was not significantly

different between patients with and without ATM-NSVs (Table II and Figure 1). The multivariable model for complete response was " unadjusted," as no other potential explanatory variable was significant in the model, and hence the lack of significance for ATM-NSV in predicting complete remission rate remained (odds ratio = 0.81, 95% CI 0.27 – 2.43; $p = 0.70$). Likewise, the lack of association between ATM-NSV and PFS (hazard ratio [HR] = 1.18, 95% CI 0.64 – 2.16; $p = 0.59$) as well as ATM-NSV and OS (HR = 0.56, 95% CI 0.27 – 1.18; $p = 0.13$) remained when adjusting for poor cytogenetics (PFS: $p = 0.04$, OS: $p = 0.009$), log-transformed WBC (OS: $p = 0.006$) and age (OS: $p = 0.01$).

Discussion

Herein we demonstrate that none of the 73 treatment-naive patients treated with fludarabine and rituximab on the CALGB 9712 study had truncating mutations of the ATM gene. Only 18 non-silent exonic variants (ATM-NSVs) in 17 patients were detected, with one variant located in the kinase domain of the ATM gene. All but two ATM-NSVs (A2308V and L15R) have been previously described (Supplementary Table 2A). The impact of some of these variants on ATM function has been inferred from indirect evidence provided by epidemiological association studies in different types of cancer [35 – 40], and remains highly controversial [41 – 45]. Infact, some of these variants show an association with reduced risk of certain types of cancer [46,47]. Our attempt to classify these ATM-NSVs as damaging or non-damaging using several *in silico* algorithms [48] produced inconsistent results for most variants, depending on the platform used (data not shown). Thus, all patients with ATM-NSVs were analyzed together.

Our data differ from other studies that have reported frequent truncating mutations and damaging missense mutations involving predominantly the kinase region of the ATM gene [23 – 25,29 – 31,49]. For example, in the studies by Pettitt *et al.*, and Austen *et al.*, truncating and exon skipping ATM mutations were detected in 12.9%, 4.4% and 12.5% of patients with CLL, respectively [30,31,49]. In terms of frequency, with sparing of the critical kinase region, our data are more similar to those reported by Yuille *et al.* and Ishibe *et al.* for familial CLL [26,28] and Lahdesmaki *et al.* for sporadic CLL [50], and support the observation of Bevan *et al.* who found no evidence of linkage between the ATM gene and familial CLL [51]. Furthermore, we identified no significant correlation between the presence of ATM-NSVs and baseline characteristics. Unlike the data of Austen *et al.*, who found a significant association between IGVH unmutated status and ATM mutations [31], the proportion of ATM-NSVs was similar between patients with mutated and unmutated IGVH (23% vs. 24%).

Although our initial hypothesis was that ATM mutations or ATM-NSVs would impact on response, we did not demonstrate an impact of ATM-NSVs on complete response rate, PFS or OS, albeit our sample size was somewhat small ($n = 73$). The number of patients subdivided by IGVH status was insufficient to derive conclusions, but suggested a trend toward adverse outcome among patients with mutated IGVH (data not shown); a large cohort of patients would be required to determine the relationship between ATM and IGVH with respect to clinical outcome. To date, few studies have examined the biologic importance of ATM mutations in CLL. Studies by Austen *et al.* ($n = 155$) identified a 12% incidence of ATM mutation, associated with shortened time to first treatment and OS [31]. A second series by this same group examined 72 patients with CLL with del(11q22.3) and found 26 (36%) who also had mutation of ATM [30]. Patients with both aberrations had a poor *in vitro* response to fludarabine, chlorambucil and cyclophosphamide based therapy as measured by DNA damage and apoptosis. Additionally, patients with both del(11q22.3) and ATM mutation had inferior survival as compared to those with just del(11q22.3). In our study ($n = 73$), no patient had a mutation of ATM, and there was no significant difference in

PFS or OS treatment outcome based upon ATM-NSV status. Only 12 patients harbored del(11q22.3), five of whom also had an ATM-NSV. All but one patient harboring del(11q22.3) progressed, and no trends were present to suggest that those with both del(11q22.3) and ATM-NSV had a worse treatment outcome as measured by PFS or OS than those with only del(11q22.3) (data not shown).

In conclusion, our relatively small study of patients with CLL treated with fludarabine and rituximab demonstrates a lower frequency of truncating ATM mutations, only one non-silent variant involving the kinase region, and no significant impact of ATM-NSV on complete response rate, PFS and OS. The small size of our study may explain the results obtained. While defective activation of this pathway has been shown to be important in predicting CLL treatment outcome, future efforts should focus on functional assays in conjunction with ATM mutational analysis. Examination of a very large study cohort will likely be required to definitively determine whether these mutations or loss of function specifically related to ATM have an impact on the treatment outcome of chemoimmunotherapy in CLL.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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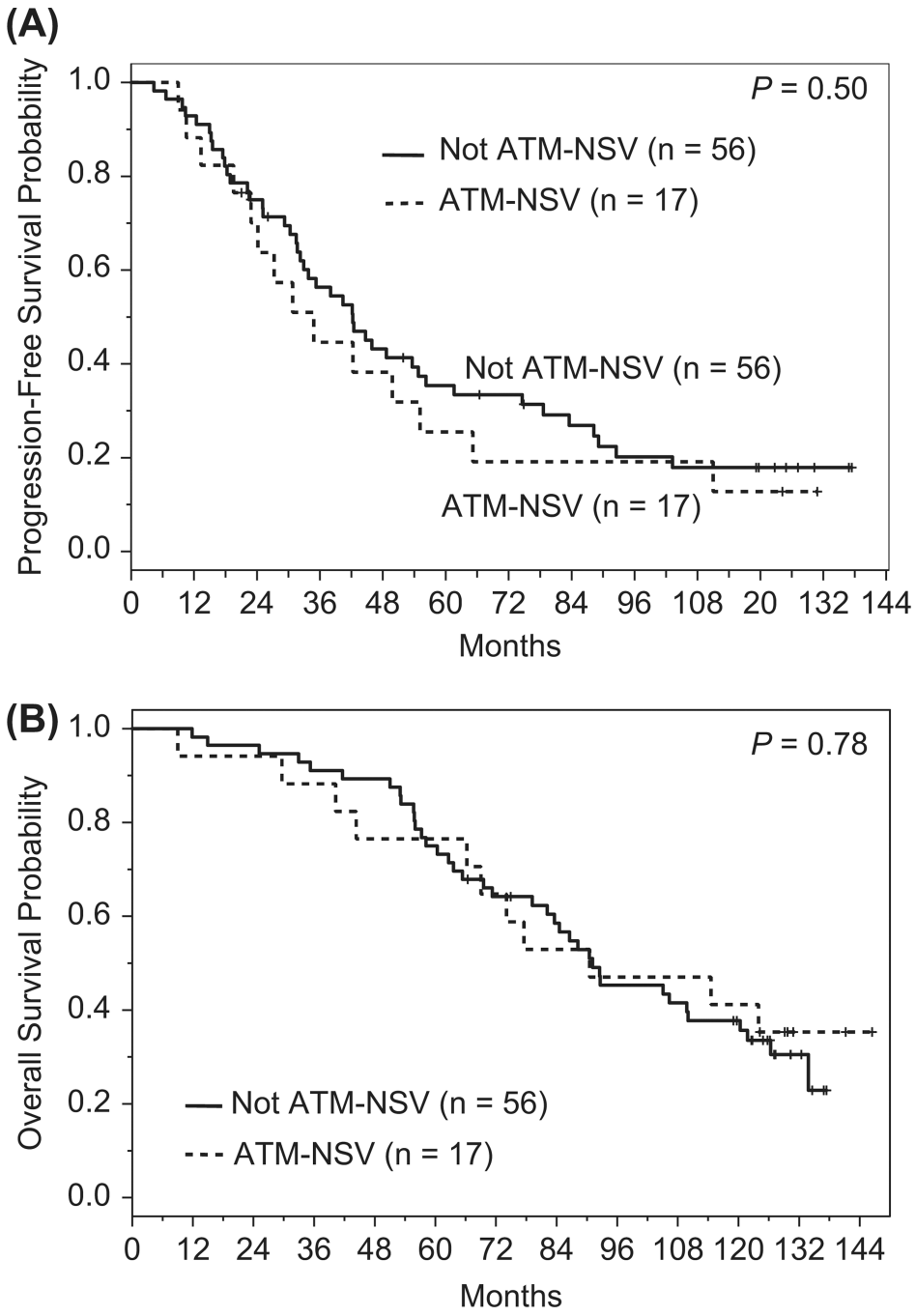

Figure 1.
Kaplan– Meier estimates for (A) progression-free survival and (B) overall survival by ATM non-silent variant (ATM-NSV) status.

Table I

Clinical and molecular characteristics of patients with CLL by ATM non-silent variant (ATM-NSV) status.

Characteristic	Overall (*n* = 73)	Not ATM-NSV (*n* = 56)	ATM-NSV (*n* = 17)	*p*-Value*
Age, years				
Median	63	63	65	0.34
Range	36–83	36–83	51–79	
Hemoglobin, g/dL				
Median	13.0	12.9	13.3	0.96
Range	1.3–16.1	1.3–15.5	8.5–16.1	
WBC, × 10^9 /L				
Median	94.1	87.0	107.4	0.14
Range	8.8–436.0	8.8–370.0	34.9–436.0	
LDH, U/L				
Median	212	212	183	0.25
Range	106–950	106–950	112–838	
Sex, *n* (%)				0.48
Male	60 (82)	47 (84)	13 (76)	
Female	13 (18)	9 (16)	4 (24)	
Rai stage, *n* (%)				1.00
I/II	44 (60)	34 (61)	10 (59)	
III/IV	29 (40)	22 (39)	7 (41)	
Hepatomegaly, *n* (%)				1.00
No	57 (83)	44 (83)	13 (81)	
Yes	12 (17)	9 (17)	3 (19)	
Splenomegaly, *n* (%)				0.77
No	26 (37)	21 (39)	5 (31)	
Yes	44 (63)	33 (61)	11 (69)	
Lymphadenopathy, *n* (%)				0.58
No	5 (7)	5 (9)	0 (0)	
Yes	68 (93)	51 (91)	17 (100)	
Interphase cytogenetics, *n* (%)				0.31
del(17p)	1 (1)	1 (2)	0 (0)	
del(11q)	12 (16)	7 (13)	5 (29)	
del(6q)	1 (1)	1 (2)	0 (0)	
trisomy 12	20 (27)	15 (27)	5 (29)	
del(13q)	23 (32)	17 (30)	6 (35)	
None of above	16 (22)	15 (27)	1 (6)	
Interphase cytogenetics, *n* (%)				0.17
Poor [del(17p), del(11q)]	13 (18)	8 (14)	5 (29)	
All others	60 (82)	48 (86)	12 (71)	
IGVH, *n* (%)				1.00
Mutated	31 (43)	24 (44)	7 (41)	

Characteristic	Overall (*n* = 73)	Not ATM-NSV (*n* = 56)	ATM-NSV (*n* = 17)	*p*-Value*
Unmutated	41 (57)	31 (56)	10 (59)	
p53 mutations, *n* (%)				1.00
No	71 (97)	54 (96)	17 (100)	
Yes	2 (3)	2 (4)	0 (0)	

CLL, chronic lymphocytic leukemia; ATM, ataxia telangiectasia mutated; WBC, white blood cells; LDH, lactate dehydrogenase; IGVH, immunoglobulin heavy chain variable gene.

*p-Value compares differences in pretreatment clinical and molecular characteristics between patients without and with ATM non-silent variants.

Table II

Treatment outcome by ATM non-silent variant (ATM-NSV) status.

Endpoint	Overall (*n* =73)	Not ATM-NSV (*n* =56)	ATM-NSV (*n* =17)	Odds/hazard ratio*	95% CI*	*p*-Value*
Induction response, *n* (%)						
Complete	19 (26)	15 (27)	4 (24)	0.84	0.24–2.99	0.79
Partial	47 (64)	37 (66)	10 (59)			
None	7 (10)	4 (7)	3 (18)			
Overall response, *n* (%)						
Complete	33 (45)	26 (46)	7 (41)	0.81	0.27–2.43	0.70
Partial	34 (47)	26 (46)	8 (47)			
None	6 (8)	4 (7)	2 (12)			
Progression-free survival (PFS)						
Median (months)	42	42	35	1.23	0.67 –2.25	0.50
PFS at 60 months, % (95% CI)	33 (22 –44)	35 (23 –48)	25 (8 –48)			
Overall survival (OS)						
Median (months)	91	91	91	0.91	0.46 –1.78	0.78
OS at 60 months, % (95% CI)	75 (64 –84)	75 (61 –84)	76 (49 –90)			

ATM, ataxia telangiectasia mutated.

*Odds ratios, 95% confidence intervals (CIs) and *p* -values are provided for comparing presence of ATM-NSV versus absence of ATM-NSV with respect to achievement of complete remission using unadjusted logistic regression models; hazard ratios, 95% CIs and *p* -values are provided for comparing presence of ATM-NSV versus absence of ATM-NSV with respect to progression-free survival and overall survival using unadjusted proportional hazards models.