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**Investigating the influence of germline *ATM* variants in chronic lymphocytic leukemia on cancer vulnerability**

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**Authors' contributions:**
*RSA and FM contributed equally as co-first authors. J.R.B. conceived and designed the study; R.S.A. and F.M. collected data and wrote the manuscript; R.S.A., F.M., and K.M. analyzed the data; S.T. performed statistical analyses; R.F., S.F., S.S., M.T., and A.P. provided administrative support (i.e., mailing the questionnaires); J.Y. designed the program to import the data; M.S.D. and J.R.B. interpreted the data; J.R.B. supervised the study. All authors approved the final version of the manuscript.

**Running head:**
Germline *ATM* variants in CLL and cancer risk

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**Abstract**

Chronic lymphocytic leukemia (CLL) patients have an increased risk of secondary cancers, along with predisposition to CLL in their relatives. We have previously identified germline *ATM* variants as associated with CLL risk; here, we present their impact on predisposition to secondary neoplasms in CLL patients and their relatives. Patients enrolled in our tissue bank who had germline *ATM* status available were mailed a questionnaire between April 2022 and May 2023. Of the 333 patients who replied to the questionnaire, 283 patients (85%) reported at least one relative with a cancer history. The prevalence of family history of B-cell lymphoproliferative disorders was significantly higher (p=0.02) in patients with germline *ATM* variants (32%) compared to those without germline *ATM* variants (21%) including familial CLL (25% vs 18%) (p=0.04). No significant difference in the prevalence of secondary cancers was found between patients with and without germline *ATM* variants (p=0.73), although the role for individual *ATM* variants in other malignancies could not be excluded given the small sample size. Time to first CLL treatment (TTFT) was shorter in patients harboring somatic *ATM* events while no difference was observed in patients with germline *ATM* variants. In conclusion, we demonstrate a higher prevalence of B-cell lymphoproliferative disorders, including familial CLL, in relatives of CLL patients carrying germline *ATM* variants. The presence of these germline variants did not impact TTFT compared to patients harboring somatic *ATM* mutations.

## Introduction

Patients with chronic lymphocytic leukemia (CLL) exhibit approximately twice the incidence of secondary malignancies compared to the general population, including common cancer types such as melanoma, breast, and prostate cancer[1–5]. Several mechanisms have been proposed to explain this association, including immunosuppression secondary to CLL, environmental exposure, and damage related to chemoimmunotherapy. Additionally, considering the role of genetic factors in the development of CLL and other cancers, shared genetic predisposition should be considered[6–8].

CLL has a strong inherited genetic component, with first-degree relatives of CLL patients having an 8.5-fold relative risk for developing CLL and other lymphoproliferative disorders[9,10]. This familial risk increases even further, up to 27.13, for relatives of CLL cases with two or more first-degree relatives with CLL [11]. However, the impact of specific ethnicity on the development of familial CLL is unknown.

While genome-wide association studies (GWAS) have identified variants explaining approximately 17% of the genetic heritability of incident CLL, the direct link between a given single nucleotide polymorphism (SNP) and CLL pathogenesis remains unidentified in almost all cases [12–14]. Candidate gene studies assessing genes implicated in CLL, particularly those involved in the DNA damage response and cell cycle pathway, have indicated the potential contribution of genes such as the Ataxia telangiectasia mutated (*ATM*) gene to CLL susceptibility[15]. Our group recently performed an unbiased exome-wide analysis that identified rare (<1%) germline variants in *ATM* and *CDK1* as associated with CLL risk[16].

The *ATM* gene, located at the 11q22.3 to q23.1 chromosome, functions as a tumor suppressor gene that dictates cellular responses during DNA damage. It is well established that somatic inactivation of *ATM* predicts worse outcomes in CLL, with a shorter time to first treatment and decreased progression-free survival after chemoimmunotherapy[17–20]. To investigate the frequency and impact of germline *ATM* variants in CLL, our group analyzed 3,128 patients who underwent clinical-grade sequencing of the entire code region of *ATM* [21]. Our analysis revealed that germline *ATM* variants are common in CLL, with rare variants present in 24% of patients, indicating a higher prevalence compared to other hematologic malignancies and

the general population without cancer [21]. The majority of these variants are missense variants with unclear functional impact on the protein, although in our earlier study we found that variants in cases were more likely to have a predicted deleterious effect on the protein. For instance, compared to wildtype *ATM*, in vitro knocked-in L2307F variant-carrying cells exhibited reduced functionality and increased susceptibility to cell death when exposed to etoposide and radiation therapy [21].

Furthermore, specific germline missense *ATM* variants have shown a strong association with an increased risk of developing breast cancer and an elevated risk for relatives who are heterozygous carriers[22,23]. A recent study also observed a significant association between germline *ATM* p.L2307F variant and lung adenocarcinoma [24,25], which happens to be the most common variant found in our cohort of CLL patients [21]. Rare germline missense *ATM* variants have also demonstrated associations with pancreatic and prostate cancer, although further research is needed to better understand this relationship[26].

Whether these missense germline *ATM* variants may predispose to other malignancies among CLL patients remains unknown and could have significant implications for cancer screening in patients and their families. This study aims to analyze the impact of germline *ATM* variants in predisposing to secondary neoplasms in CLL patients and their relatives.

## Methods

### Patients' Population

Patients were eligible to participate in this study if they had a confirmed diagnosis of CLL or SLL that met International Workshop on CLL criteria [27], had NGS that assessed germline *ATM* status, and were included in the internal Dana-Farber Cancer Institute CLL Database. These patients were mailed a questionnaire between April 2022 and May 2023. The questionnaire assessed: demographics; personal and family history of any cancer; non-medical radiation and Agent Orange exposure; ataxia-telangiectasia syndrome. European ancestry was categorized according to the United Nations Geoscheme, one of the systems used to classify countries into subregional groups [28].

Information collected from our database included biological characteristics including FISH, immunoglobulin heavy chain variable region (IGHV), and *TP53* status, data on CLL history at last follow-up, and treatment information. This information was also collected for patients who did not reply to the questionnaires.

Ethical approval was obtained from the Dana-Farber Cancer Institute Institutional Review Board, and all patients provided written informed consent prior to sample and data collection.

Patients' Classification

Enrolled patients were stratified into four groups, based on *ATM* mutational status (germline and somatic) and somatic del(11q). *ATM* status was defined through direct germline sequencing of saliva or by inference according to the hierarchical algorithm we have recently published [21]. Patients were initially classified into four groups for the demographic and clinical characteristic analysis as follows: Group-1, germline *ATM* variants alone; Group-2, germline *ATM* with somatic *ATM* variants and/or del(11q); Group-3, somatic *ATM* aberration alone (including del(11q)); and Group-4, no *ATM* aberration **(Figure 1)**. To analyze the cancer prevalence, we combined Group-1 and Group-2 vs Group-3 and Group-4 into two groups based on the presence or absence of germline *ATM* variants.

Endpoints

The primary endpoint was to assess whether patients with germline *ATM* variants and their relatives had a higher prevalence of secondary tumors compared with those without germline *ATM* variants, including evaluating the frequency of familial CLL and other lymphoproliferative disorders in the two groups. Key secondary endpoints included analyzing the age at diagnosis of second cancer, examining the impact of ethnicity on the development of familial B-cell lymphoproliferative disorders, and analyzing the impact of *ATM* germline variants on Time to First Treatment (TTFT).

Statistics

The patient characteristics were described using frequency tables for qualitative variables and mean and range for quantitative variables. The associations between clinical-biological

parameters and received treatment regimens were analyzed using the Chi-square or Fisher's exact test for qualitative variables, and the Wilcoxon or Kruskal-Wallis test for quantitative variables. The Dwass-Steel-Critchlow-Fligner test was utilized to assess post-hoc pairwise comparisons.

TTFT was calculated from the date of diagnosis until the date of first CLL treatment; untreated subjects at last follow-up were censored. The probabilities of TTFT were estimated using the Kaplan-Meier method; the Cox proportional hazard regression test was used to compare the different groups of patients. Confidence intervals were calculated at 95%, all tests were two-tailed, and differences with $p < 0.05$ were considered statistically significant. For the analyses, Jamovi[29] software was used.

## Results

### Patients

From April 2022 to May 2023, a total of 587 CLL patients received a questionnaire by mail, and 333 patients (57%) replied. The patients who did not reply to the questionnaire had a younger age at CLL diagnosis (57 years as compared to 60 years, p=0.03) and a higher prevalence of unknown ethnicity (10% as compared to 1%, p <0.001). However, the remaining clinical characteristics were similar between patients who responded to questionnaries and those who did not (Supplemental Table 1).

The median age of our cohort was 60 years (range, 28 to 89). A total of 189 patients (57%) were male, and 327 patients (98.2%) self-identified as non-Hispanic. European ancestry was self-reported by 206 patients (62%), with the majority being descendants from Northern Europe. Further details on European ancestry can be found in the Supplementary Appendix (Supplemental Table 2). In addition, 7% of the patients identified themselves as of Ashkenazi Jewish origin. Unmutated IGHV status was observed in 152 (46%) of patients, and 48 (14%) carried 17p deletion, *TP53* mutation, or both. Further baseline demographic and biological characteristics are available in **Table 1**.

Regarding *ATM* status and/or 11q deletion, 85 patients (26%) had at least one germline *ATM* variant, 22 patients (7%) had at least one somatic *ATM* mutation, and 51 patients (15%) had 11q deletion. Among the 85 patients with germline *ATM* variants, 22 patients had both germline

and somatic *ATM* variants and/or 11q deletion. Additionally, 22 patients had only somatic *ATM* mutations, 19 patients had 11q deletion without *ATM* mutations, and 207 patients had neither *ATM* mutations nor 11q deletion. The median time between CLL diagnosis and mutation analysis for patients harboring somatic *ATM* mutation was 28.5 months (range, 0 to 207), with 19 patients (43.2%) undergoing mutational analysis within the first year after CLL diagnosis.

Among the 85 patients with germline *ATM* variants, 58 variants were classified as benign, 13 as probably benign, 7 as VUS, 3 as probably pathogenic, and 4 as pathogenic acording to the ACMG classification [30]. The *ATM* somatic mutations were predicted to be VUS (11 patients), pathogenic (5 patients) or likely pathogenic (6 patients) **(Figure 2)**. All germline *ATM* variants classified as benign and likely benign were considered rare (present at an overall population allelic frequency of < 1%)[31].

Based on the *ATM* mutational status, 63, 22, 41 and 207 patients were classified as Group-1, Group-2, Group-3, and Group-4, respectively **(Figure 1)**. The baseline demographic and clinical characteristics were balanced among the four groups except for IGHV status **(Table 1)**. Unmutated IGHV was more common in patients carrying somatic *ATM* variants (33% vs 86% vs 88% vs 37% in group 1 vs 2 vs 3 vs 4, respectively; $p < 0.001$).

Second malignancy history

One hundred and sixty-four patients (49%) had a history of an additional non-CLL neoplasm, and 221 cancers were reported. Excluding patients with only non-melanoma skin cancer (NMSC), the prevalence was reduced to 31% (104 patients).

The most prevalent types of cancers were as follows: NMSC (99, 30%), prostate (27, 14%), breast (19, 13%), melanoma (27, 8%), thyroid (7, 2.1%), and colon (5, 2%). The prevalence of other lymphomas, excluding Richter's transformation, was 1%. The median number of secondary cancers per patient was 1 (range, 1 to 4). For patients with two or more secondary malignancies, the most common combinations were NMSC with melanoma (17, 5.1%) and, for male patients, NMSC with prostate cancer (12, 3.6%).

Of the 134 patients for whom age at secondary malignancy was available, 54 patients (40%) had developed the secondary cancer prior to CLL diagnosis, while 80 patients (60%) were diagnosed with the secondary cancer following their CLL diagnosis. With a median follow-up between CLL diagnosis and response to the questionnaire of 7.6 years (range, 2.5 to 43.7), the

median time to the development of secondary cancer after CLL diagnosis was 6 years (range, 1-37 years). Notably, 133 patients (39.9%) did not reach a follow-up period of 6 years.

Patients who developed a secondary cancer after their CLL diagnosis were younger at the time of CLL diagnosis than those who had a secondary cancer before their CLL diagnosis (median age 59 years versus 66 years, respectively, $p < 0.001$). Additionally, these patients were older at the time of secondary cancer diagnosis compared to those who developed secondary cancer before their CLL diagnosis (median age 67 versus 57 years, respectively, $p < 0.001$). However, no significant difference was observed in the subtype of secondary cancer, although all patients with colon cancer were diagnosed after their CLL diagnosis.

Among the 80 patients diagnosed with the secondary cancer after CLL diagnosis, 56 patients (70%) had received at least one line of treatment, with 32 patients (40%) treated before the secondary cancer diagnosis. Of these 32 treated patients, 17 received chemoimmunotherapy, 12 were treated with targeted therapy and 3 patients were treated with both chemoimmunotherapy and targeted therapy.

Familial patterns and inheritance

Within the total cohort, 283 patients (85%) reported a family history of cancer, with a median of 2 relatives affected (range, 1 to 17), including first-, second- and third-degree relatives. The prevalence of B-cell lymphoproliferative disorders was significantly higher ($p = 0.02$) in relatives of patients with germline *ATM* variants (26, 31%) compared to those without (47, 19%). When specifically analyzing patients with one or more relatives diagnosed with CLL, the incidence of familial CLL was also significantly higher in patients with germline *ATM* variants (21, 25%) compared to those without germline *ATM* variants (37, 15%) ($p = 0.04$) **(Figure 3A and 3B)**.

While the prevalence of germline *ATM* variants was similar between patients of Ashkenazi Jewish and non-Ashkenazi Jewish ethnicity (8, 9.4%, in Ashkenazi Jewish versus 15, 6%, in non-Ashkenazi Jewish, $p = 0.29$), the prevalence of familial CLL showed a strong association with Ashkenazi Jewish origin. Among patients of Ashkenazi Jewish origin, there was a 39% prevalence of familial CLL, compared to 16% in patients not of Ashkenazi Jewish origin ($p = 0.004$) **(Figure 3C)**.

No difference in the incidence of solid malignancies was found among the relatives of patients with germline *ATM* and without. No patients reported a family or personal history of ataxia-telangiectasia syndrome.

Correlation between *ATM* variants and secondary tumors

Comparing patients with germline *ATM* variants (N=85) to those without germline *ATM* variants (N=248), overall, no significant difference was found in the prevalence of secondary cancers (p = 0.98). More in detail, no difference was found when comparing the prevalence of NMSC, prostate cancer, breast cancer, melanoma, colorectal cancer, and other solid tumors **(Figure 4A)**. Additionally, the median age of secondary cancer onset was also similar between groups, with a median age of 63 years-old for germline *ATM* variants present and 64 years-old for germline *ATM* variants absent (p = 0.28) **(Figure 4B)**. In the 80 patients who developed a secondary cancer after their CLL diagnosis, no significant differences were observed between those with germline *ATM* variants and those without, in terms of age at secondary cancer diagnosis or the different cancer subtypes.

Time to first treatment analysis

Overall, 199 (60%) patients received at least one line of CLL therapy, with a median number of 1 line (range, 1 to 7). While no difference in the median number of treatment lines was observed among the four groups (p = 0.467), a greater number of patients carrying somatic *ATM* mutation and/or 11q deletion (Group-2 and Group-3) received at least a first line treatment compared to the patients in Group-1 and Group-4 (86% vs 80% vs 57% vs 54%, respectively; p<0.001) **(Figure 5A)**.

Compared to patients without any *ATM* aberration, TTFT was shorter in patients with somatic *ATM* events, whereas no difference was observed between patients with germline *ATM* variants (median TTFT: 82, 59, 52, 90 months for Group-1, Group-2, Group-3, and Group-4, respectively, p=0.01) **(Figure 5B)**.

After excluding the 54 patients with a secondary cancer before their CLL diagnosis, no difference in the incidence of secondary cancer was observed between those who received CLL treatment and those who did not, even after stratifying by patients who received three or more lines of CLL therapy.

## Discussion

The higher risk of developing secondary malignancies in CLL patients is a growing concern, especially given the improved outcomes associated with new available therapies [3–5,27]. The underlying mechanisms that lead to predisposition for secondary tumors remains poorly understood. Despite the known association between *ATM* germline variants and certain cancers [23–25,32,33], our study did not identify a significant association between germline *ATM* variants collectively and a higher prevalence of secondary tumors in CLL patients. However, our results do not rule out a role for individual variants in other malignancies, as already described for *ATM* L2307F for example. The inclusion of both benign and pathogenic variants in our analysis was necessary due to the low population incidence of any single variant, and the overall small sample size. However, this approach, combined with the short follow-up for more than one-third of the patients, may have masked associations with pathogenic variants because of their low frequency.

Furthermore, the outcome of our investigation holds significant implications within the context of CLL and its intricate genetic background. Notably, our data indicate an increased occurrence of B-cell lymphoproliferative disorders, particularly familial CLL, among the family members of CLL patients harboring germline *ATM* variants. This finding extends ours and others' prior observations that *ATM* variants are enriched among CLL patients themselves and highlights once again the possible role of germline *ATM* variants in cancer development [15,16,21,34].

Indeed, our results suggest that the familial clustering of CLL must consider the significant contribution of germline genetic anomalies. These specific genetic factors not only impact an individual's odds of developing CLL but also increase the susceptibility of their relatives who carry these variants to develop cancer. We found a higher prevalence of familial CLL among patients of Ashkenazi Jewish origin, consistent with a study from Israel that also identified an association between Ashkenazi Jewish descent and familial CLL [35] which warrants further investigation.

Although germline *ATM* variants seem to play a role in the familial aggregation of CLL, they do not impact on TTFT compared to patients harboring somatic *ATM* mutations. The negative influence of somatic *ATM* mutations is confirmed here by their association with the progression of CLL and the subsequent need for patients to be treated [17,36]. While germline *ATM* variants are associated with impact on cancer susceptibility, somatic *ATM* mutations appear to

exhibit closer links to CLL progression. This finding could be linked to the typically more pathogenic nature of the somatic mutations observed or could suggest distinct roles of *ATM* variants at different stages of disease evolution, from initial and familial predisposition to disease progression. [37].

Our study has several limitations inherent to its retrospective design. The analysis was conducted using data from a single center, and we acknowledge the possibility of selection bias. Additionally, guaranteed time bias is a limitation due to the selection of patients for sequencing and the survival time until they responded to the questionnaire. The number of patients included in this cohort may also not have been sufficient to detect a significant difference in the prevalence of second tumors between the two groups. It is important to recognize that these limitations may affect the generalizability of our findings, and larger studies are needed to confirm our results. Larger studies are also needed to be able to dissect the impact of individual *ATM* variants, which undoubtedly vary in their functional impact on the protein and on genetic susceptibility to different cancers.

In conclusion, the augmented prevalence of B-cell lymphoproliferative disorders, particularly familial CLL, among relatives of CLL patients carrying germline *ATM* variants underscores the relevance of genetic components in familial CLL predisposition[16]. Although these variants don't appear to impact TTFT in this dataset, their presence adds to our comprehension of the hereditary background of CLL. Continued research is needed to better understand the exact mechanisms through which germline *ATM* variants individually and collectively impact on familial risk, and to comprehensively understand the wider implications of these variants within the context of CLL and related conditions.

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| Table 1: Demographic and Clinical Characteristic of the Patients[#] | | | | | |
|---|---|---|---|---|---|
| **Characteristic** | **Group 1 (N = 63)** | **Group 2 (N = 22)** | **Group 3 (N = 41)** | **Group 4 (N = 207)** | **p value** |
| **Age at diagnosis** | | | | | p=0.48 |
| Median (range) – years | 60 (40-75) | 58 (30-71) | 61 (34-87) | 61 (28-89) | |
| Distribution – no (%) | | | | | |
| ≤ 55 years | 22 (35) | 10 (45) | 12 (29) | 70 (34) | |
| **Sex – n (%)** | | | | | p=0.20 |
| Male | 33 (52) | 14 (64) | 29 (71) | 113 (55) | |
| Female | 30 (48) | 8 (36%) | 12 (29) | 94 (45) | |
| **Race – n (%)** | | | | | p=0.32 |
| White | 63 (100) | 21 (95) | 40 (98) | 205 (99) | |
| Black or African American | - | - | - | 2 (1) | |
| Asian | - | 1 (5) | - | - | |
| Native Hawaiian/Pacific Island | - | - | 1 (2) | - | |
| **Ethnicity – n (%)** | | | | | p=0.48 |
| Not Hispanic or Latino | 63 (100) | 22 (100) | 41 (100) | 201 (97) | |
| Hispanic or Latino | - | - | - | 4 (2) | |
| Unknown | - | - | - | 2 (1) | |
| **Ashkenazi Jewish ethnicity – n (%)** | | | | | p=0.20 |
| Yes | 4 (6) | 4 (18) | 2 (5) | 13 (6) | |
| No | 58 (92) | 18 (82) | 38 (93) | 191 (92) | |
| **Unknown** | **1 (2)** | **-** | **1 (2)** | **3 (2)** | |
| **Non-medical radiation exposure – n (%)** | | | | | p=0.18 |
| Yes | 2 (3) | - | 2 (5) | 17 (8) | |

| | | | | | |
|---|---|---|---|---|---|
| No | 61 (97) | 22 (100) | 39 (95) | 188 (91) | |
| Unknown | - | - | - | 2 (1) | |
| **Agent orange exposure – n (%)** | | | | | p=0.13 |
| Yes | - | - | - | 7 (3) | |
| No | 63 (100) | 22 (100) | 41 (100) | 198 (96) | |
| Unknown | - | - | - | 2 (1) | |

**Table 1: Demographic and Clinical Characteristic of the Patients (Continuation)[#]**

| **Characteristic** | **Group 1 (N = 63)** | **Group 2 (N = 22)** | **Group 3 (N = 41)** | **Group 4 (N = 207)** | **p value** |
|---|---|---|---|---|---|
| **European ancestry – n (%)** | | | | | p=0.37 |
| Yes | 41 (65) | 11 (50) | 29 (71) | 125 (60) | |
| No | 22 (35) | 11 (50) | 12 (29) | 82 (40) | |
| **Richter's transformation – n (%)** | | | | | p=0.83 |
| Yes | 2 (3) | - | - | 7 (3) | |
| No | 61 (97) | 22 (100) | 41 (100) | 200 (97) | |
| **IGHV status – n (%)** | | | | | p<0.001 |
| Mutated | 32 (51) | 2 (9) | 5 (12) | 108 (52) | |
| Unmutated | 21 (33) | 19 (86) | 36 (88) | 76 (37) | |
| Unknown | 10 (16) | 1 (5) | - | 23 (11) | |
| **Del(17p) and/or TP53 aberration – n (%)** | | | | | p=0.80 |
| Yes | 8 (13) | 3 (14) | 4 (10) | 33(16) | |
| No | 55 (87) | 19 (86) | 37 (90) | 172 (83) | |
| Unknown | - | - | - | 2 (1) | |
| **Year of CLL diagnosis** | | | | | p=0.42 |
| Median (range) - year | 2014 | 2013 | 2016 | 2015 | |

| | | | | |
|---|---|---|---|---|
| | (1996-2019) | (1980-2019) | (2001-2019) | (1980-2019) |

[#]All demographics are self-reported. Patients categorized as "*no European ancestry*" self-reported as Americans. Group 1: *ATM* germline variants alone; Group 2: *ATM* germline with somatic *ATM* variants and/or del(11q); Group 3: *ATM* somatic aberration alone (including del(11q)); and Group 4: no *ATM* aberration. IGHV: immunoglobulin heavy chain variable region gene.

**Figure Legends**

**Figure 1: Classification of patients according to germline and somatic *ATM* status.** CLL: chronic lymphocytic leukemia.

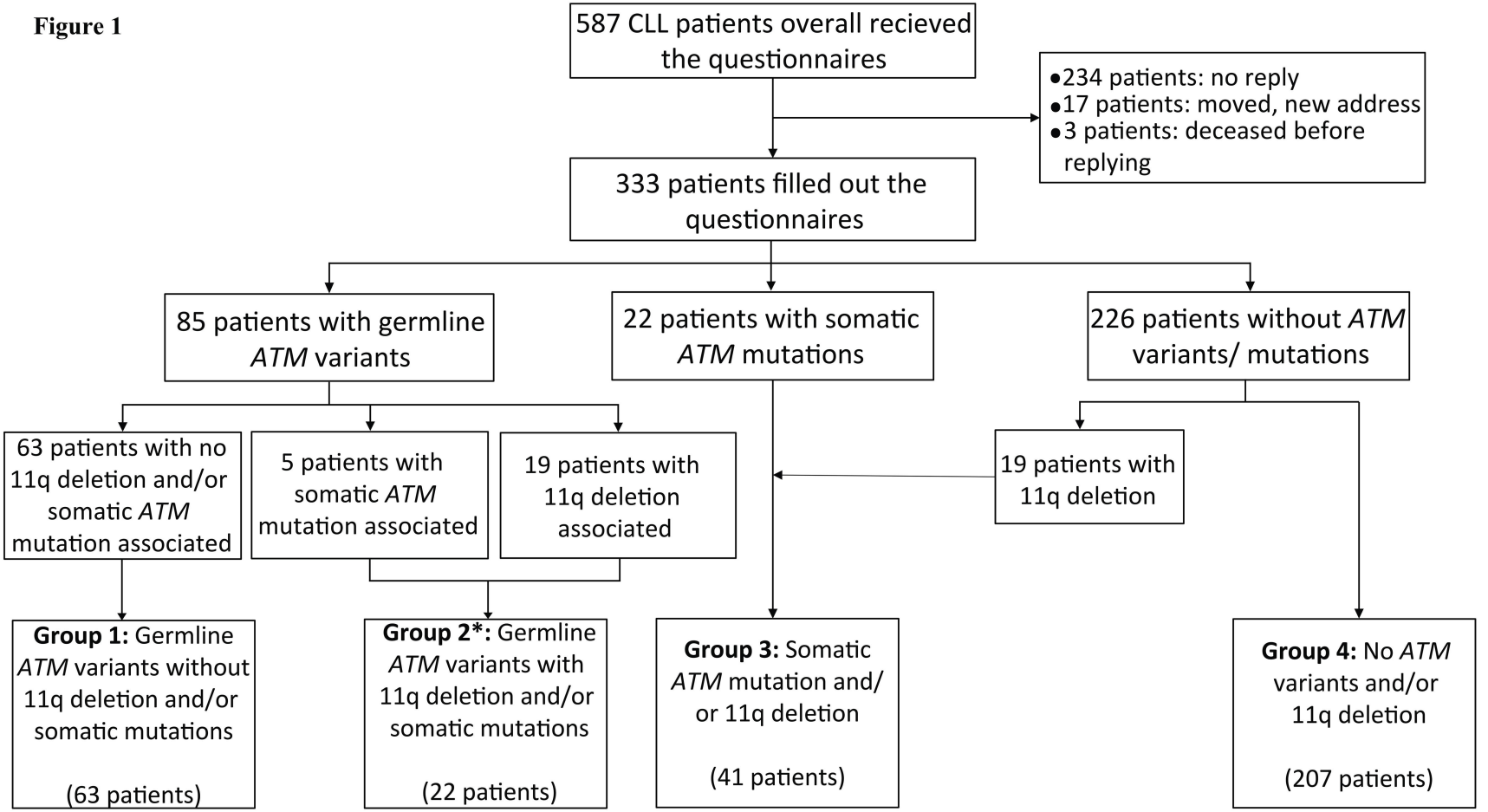
**Figure 2: Characterization of *ATM* variants identified in our cohort. (A)** Pie chart demonstrating the prevalence of *ATM* variants in the whole cohort along with the frequencies of germline *ATM* variants and somatic *ATM* mutation. The germline *ATM* variants group includes 6% of patients who also harbor somatic *ATM* mutation. No germline *ATM* variant is included in the somatic *ATM* mutation group. **(B)** Bar chart with the predicted pathogenicity of each variant according to American College of Medical Genetics and Genomics (ACMG) classification rules, broken down by germline or somatic status. VUS: variant of uncertain significance.

**Figure 3: Comparison of prevalence of malignancies in relatives of patients with CLL. (A)** Prevalence of familial B-cell lymphoproliferative disorders in patients with and without germline *ATM* variant. **(B)** Prevalence of familial CLL in patients with and without germline *ATM* variant. (C) Prevalence of familial CLL in patients with and without Ashkenazi Jews origin. CLL: chronic lymphocytic leukemia. * $p<0.05$; ** $p<0.01$.

**Figure 4: Comparison of second malignancies between patients with and without germline *ATM* variants. (A)** Bar chart showing the frequency of second tumor divided in different subtypes. For prostate cancer, the prevalence was evaluated among male patients; for breast cancer, the prevalence was evaluated among female patients (one male patient with breast cancer was excluded from the analysis). **(B)** Age at second tumor diagnosis in patients with and without germline *ATM* variants. CLL: chronic lymphocytic leukemia; NMSC: nonmelanoma skin cancer.

**Figure 5: Patients treated for CLL. (A)** Percentage of patients who were treated in each group, according to iwCLL (international workshop on chronic lymphocytic leukemia) criteria. The pairwise comparison using the Dwass-Steel-Critchlow-Fligner test showed a difference between Group-2 and Group-4 ($p=0.018$) and between Group-3 and Group-4 ($p=0.009$). Between Group-1 versus Group-2 and Group-3, statistical significance was almost achieved ($p=0.067$ and $p=0.068$, respectively). No differences were observed in the pairwise comparison between Group-1 and Group-4 ($p=0.969$) and Group-2 and Group-3 ($p=0.938$). **(B)** Time to first treatment for each group. Group-1: Germline *ATM* variants without 11q deletion and/or somatic variants; Group-2: Germline *ATM* variants with 11q deletion and/or somatic variants; Group-3: Somatic *ATM* variants and/or 11q deletion; Group-4: No *ATM* mutations and/or 11q deletion. TTFT: time to first treatment. * $p<0.05$; ** $p<0.01$.

**Figure 1**




* 2 patients with germline *ATM* variants have both somatic *ATM* mutation and 11q deletion

**Figure 2**

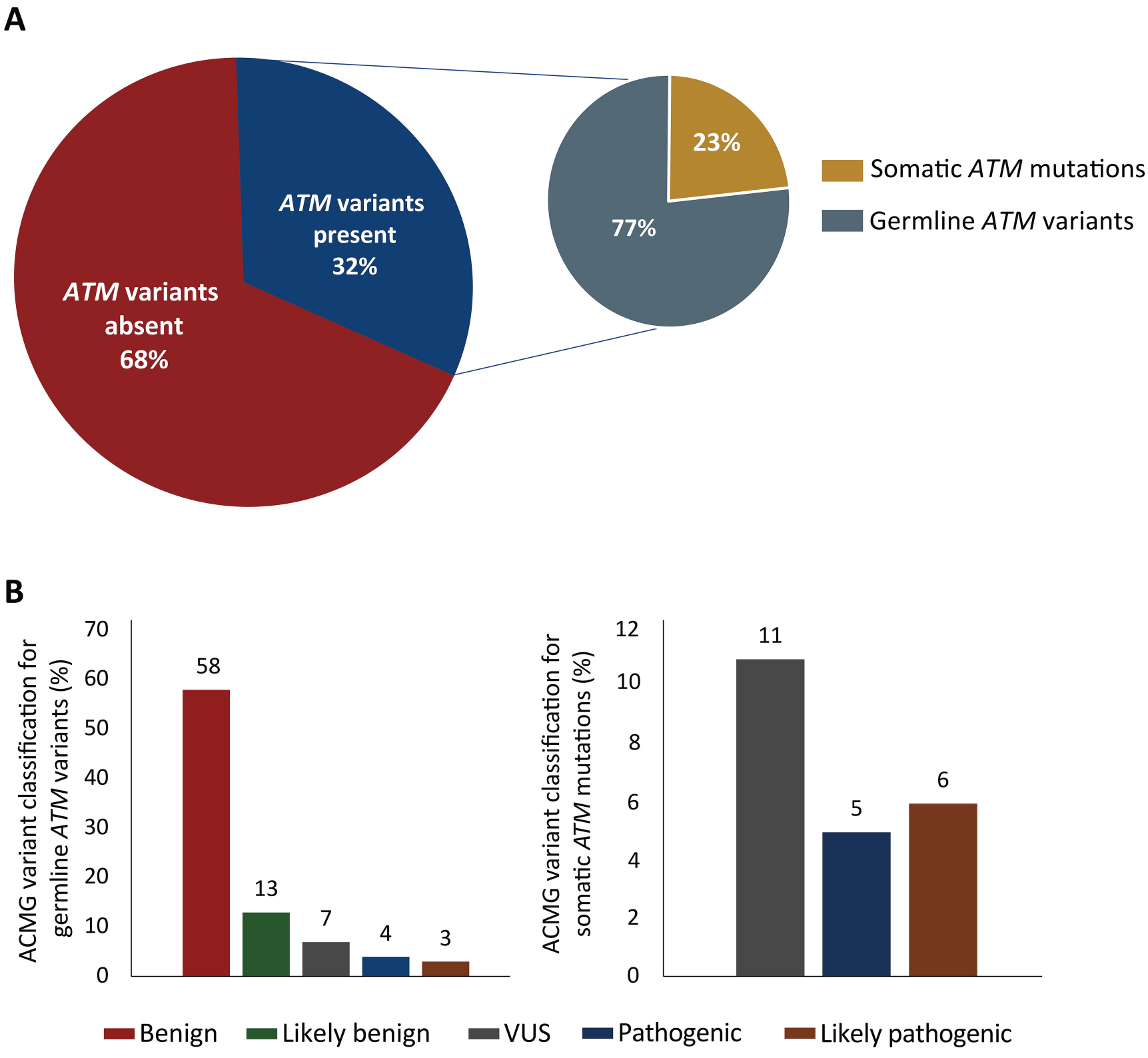

A
ATM variants absent 68%
ATM variants present 32%
23%
77%
Somatic ATM mutations
Germline ATM variants
B
ACMG variant classification for germline ATM variants (%)
70
60
50
40
30
20
10
0
58
13
7
4
3
ACMG variant classification for somatic ATM mutations (%)
12
10
8
6
4
2
0
11
5
6
Benign
Likely benign
VUS
Pathogenic
Likely pathogenic

**Figure 3**

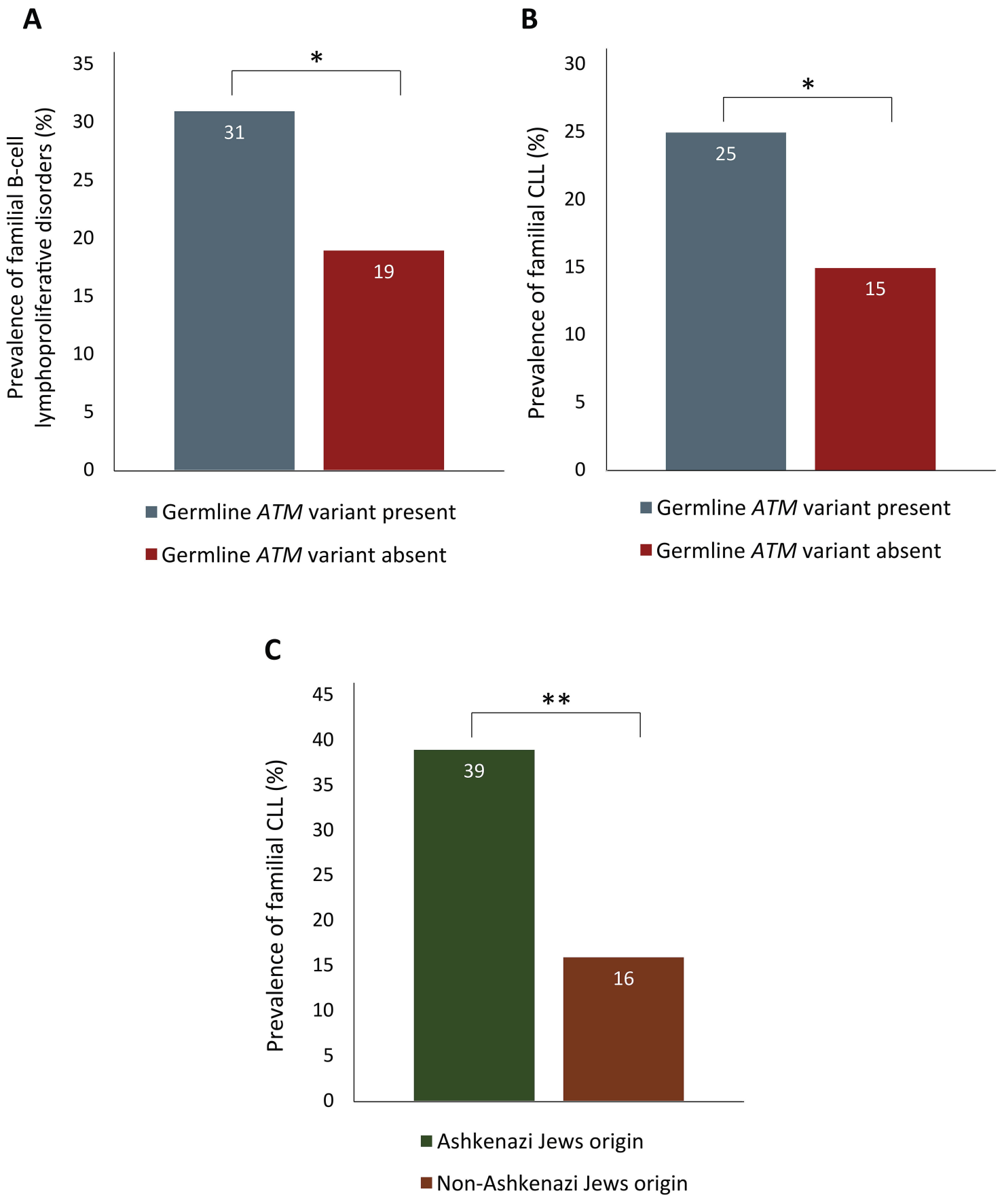

A
Prevalence of familial B-cell lymphoproliferative disorders (%)
35
30
25
20
15
10
5
0
*
31
19
Germline ATM variant present
Germline ATM variant absent
B
Prevalence of familial CLL (%)
30
25
20
15
10
5
0
*
25
15
Germline ATM variant present
Germline ATM variant absent
C
Prevalence of familial CLL (%)
45
40
35
30
25
20
15
10
5
0
**
39
16
Ashkenazi Jews origin
Non-Ashkenazi Jews origin

**Figure 4**

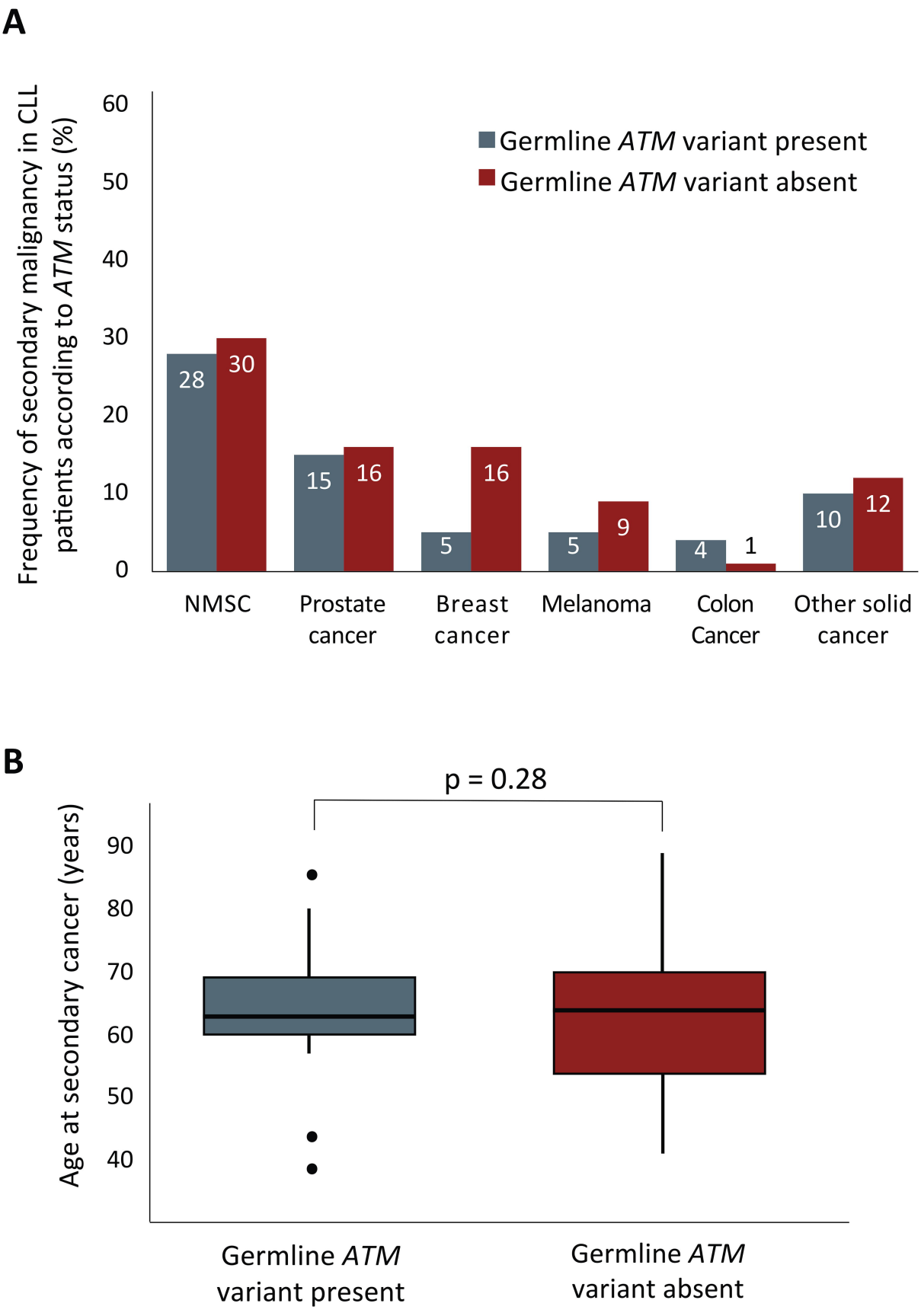
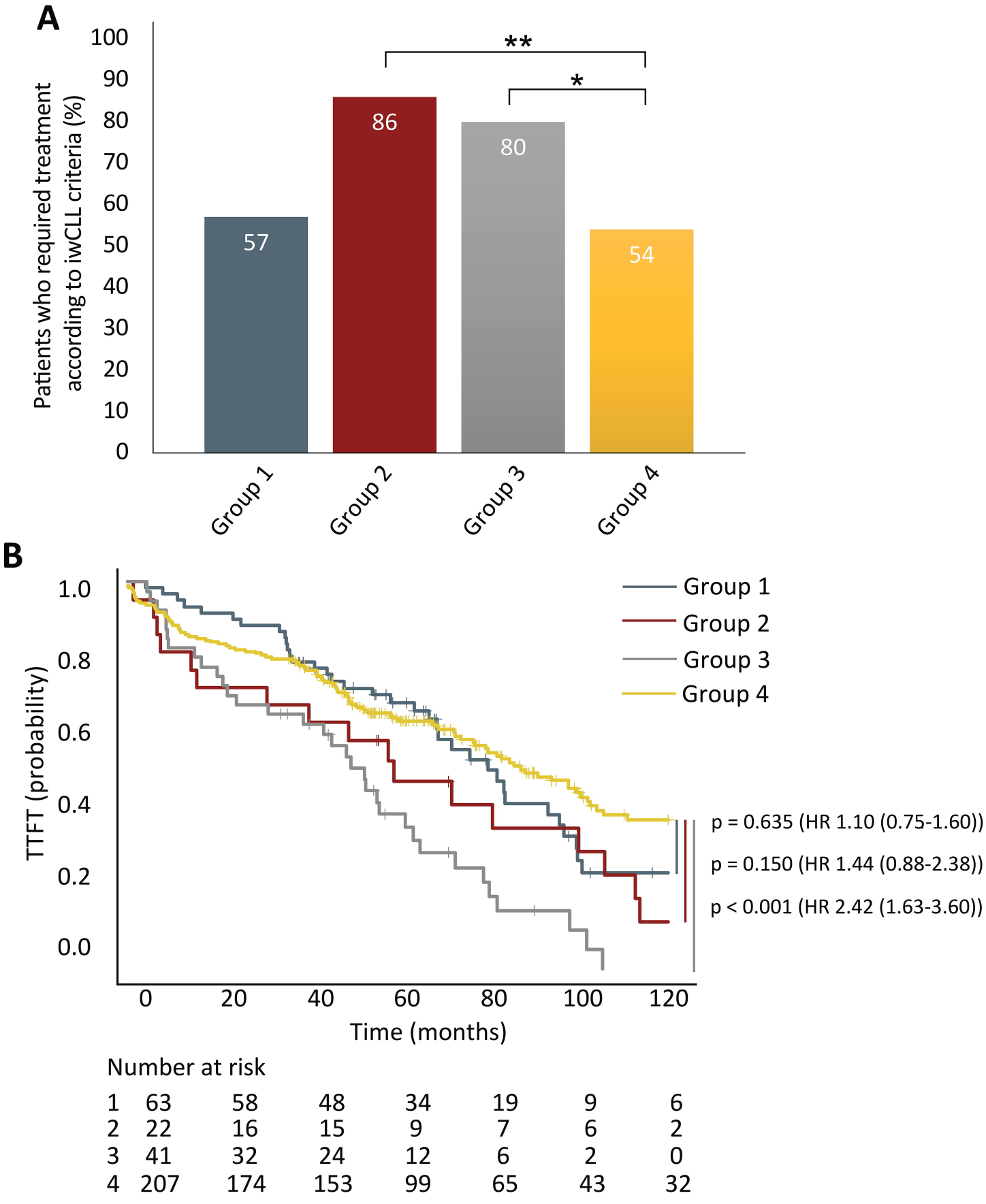

A
Frequency of secondary malignancy in CLL patients according to ATM status (%)
60
50
40
30
20
10
0
Germline ATM variant present
Germline ATM variant absent
28
30
15
16
5
16
5
9
4
1
10
12
NMSC
Prostate cancer
Breast cancer
Melanoma
Colon Cancer
Other solid cancer
B
p = 0.28
Age at secondary cancer (years)
90
80
70
60
50
40
Germline ATM variant present
Germline ATM variant absent

**Figure 5**


A
Patients who required treatment according to iwCLL criteria (%)
100
90
80
70
60
50
40
30
20
10
0
**
*
86
80
57
54
Group 1
Group 2
Group 3
Group 4
B
TTFT (probability)
1.0
0.8
0.6
0.4
0.2
0.0
Group 1
Group 2
Group 3
Group 4
p = 0.635 (HR 1.10 (0.75-1.60))
p = 0.150 (HR 1.44 (0.88-2.38))
p < 0.001 (HR 2.42 (1.63-3.60))
0
20
40
60
80
100
120
Time (months)
Number at risk
1 63 58 48 34 19 9 6
2 22 16 15 9 7 6 2
3 41 32 24 12 6 2 0
4 207 174 153 99 65 43 32

| Supplemental Table 1: Demographic and Clinical Characteristic of all Cohort[#] | | | |
|---|---|---|---|
| **Characteristic** | **Patients who replied to the questionnaires (N = 333)** | **Patients who did not reply to the questionnaires (N = 254)** | **p value** |
| **Age at diagnosis** | | | p=0.03 |
| Median (range) – years | 60 (28-89) | 57 (23-89) | |
| Distribution – no (%) | | | |
| ≤ 55 years | 114 (34) | 110 (43) | |
| **Sex – n (%)** | | | p=0.09 |
| Male | 189 (57) | 162 (64) | |
| Female | 144 (43) | 92 (36) | |
| **Ethnicity – n (%)** | | | <0.001 |
| Not Hispanic or Latino | 327 (98) | 220 (87) | |
| Hispanic or Latino | 4 (1) | 6 (2) | |
| Unknown | 2 (1) | 26 (10) | |
| **Richter's transformation – n (%)** | | | 0.68 |
| Yes | 9 (3) | 10 (4) | |
| No | 324 (97) | 244 (96) | |
| ***ATM* mutation – no (%)** | | | 0.06 |
| Germline | 85 (25) | 58 (23) | |
| Somatic | 22 (7) | 7 (3) | |
| Neither germline or somatic | 226 (68) | 189 (74) | |
| **IGHV status – n (%)** | | | 0.36 |
| Mutated | 147 (44) | 120 (47) | |
| Unmutated | 152 (46) | 102 (40) | |
| Unknown | 34 (10) | 32 (13) | |
| **Del(17p) and/or *TP53* aberration – n (%)** | | | 0.09 |
| Yes | 48 (14) | 45 (18) | |
| No | 283 (85) | 203 (80) | |
| Unknown | 2 (1) | 6 (2) | |
| **Year of CLL diagnosis** | | | 0.04 |
| Median (range) - year | 2015 (1980-2019) | 2016 (1995-2019) | |

[#]All patients who were sent questionnaires. CLL: chronic lymphocytic leukemia; IGHV: immunoglobulin heavy chain variable region gene.

| Supplemental Table 2: Geographic Regions of Europe* | |
|---|---|
| **Geographic Regions** | **Patients (N = 206)** |
| Northern | 61 |
| Southern | 30 |
| Western | 14 |
| Eastern | 23 |
| Northern + Western | 38 |
| Northern + Southern | 13 |
| Northern + Eastern | 11 |
| Western + Eastern | 6 |
| Southern + Eastern | 4 |
| Western + Southern | 2 |
| Three or more Geographic Regions | 4 |

* United Nations geoscheme subregions of Europe: Eastern Europe: Belarus, Bulgaria, Czechia, Hungary, Poland, Republic of Moldova, Romania, Russian Federation, Slovakia, Ukraine; Northern Europe: Aland Islands, Denmark, Estonia, Faroe Islands, Filand, Guernsey, Iceland, Ireland, Isle of Man, Jersey, Latvia, Lithuania, Norway, Svalbard and Jan Mayen Island, Sweden, United Kindom of Great Britain and Northern Ireland; Southern Europe: Albania, Andorra, Bosnia and Herzegovina, Croatia, Gibraltar, Greece, Holy See, Italy, Malta, Montenegro, North Macedonia, Portugal, San Marino, Serbia, Slovenia, Spain; Western Europe: Austria, Belgium, France, Germany, Liechtenstein, Luxembourg, Monaco, Netherlands, Switzerland, Landlocked Developing Countries (LLDC), Least Developed Countries (LDC).