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Association between immunoglobulin heavy-chain variable region mutational status and isolated favorable baseline genomic aberrations in chronic lymphocytic leukemia

Jose D. Sandoval-Sus[a,*], **Julio C. Chavez**[b,*], **Samir Dalia**[c], **Syeda Mahrukh Hussnain Naqvi**[d], **Chetasi Talati**[a], **Lisa Nodzon**[b], **Mohamed A. Kharfan-Dabaja**[e], **Javier Pinilla-Ibarz**[b]

[a]H. Lee Moffitt Cancer Center and Research Institute, University of South Florida, Tampa, FL, USA

[b]Department of Malignant Hematology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA

[c]Mercy Clinic Oncology and Hematology, Joplin, MO, USA

[d]Department of Biostatistics and Bioinformatics, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA

[e]Department of Blood and Marrow Transplantation, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA

Abstract

Immunoglobulin heavy-chain variable region (*IGHV*) mutational status and karyotype abnormalities are important prognostic factors in chronic lymphocytic leukemia (CLL). The goal was to assess the impact of *IGHV* in CLL patients with isolated favorable genetic aberrations (del13q, trisomy 12, or negative fluorescence *in situ* hybridization [FISH]). We studied 273 CLL patients with both *IGHV* mutational status and cytogenetic information: 145 with isolated del13q 49 with sole trisomy 12 and 79 with negative FISH. After a median follow-up of 7.8 years, patients with del13q-unmutated *IGHV* had a shorter time to first treatment (TFT) (2.98 vs. 17.44 years; $p<.001$) and shorter overall survival (10.45 years vs. not reached; $p=.0026$). Patients with negative FISH-unmutated *IGHV* had shorter TFT ($p=.02$) (3.10 vs. 9.75 years, $p=.053$). *IGHV* status did not influence clinical outcomes in trisomy 12 CLL. In conclusion, *IGHV* mutational status shows prognostic impact in CLL patients with good prognosis genomic features.

Keywords

Chronic lymphocytic leukemia; *IGHV*; genomic abnormalities; survival

CONTACT Julio C. Chavez, julio.c.chavez@moffitt.org, Department of Malignant Hematology, Moffitt Cancer Center, 12902 Magnolia Drive FOB3-HEME, Tampa, FL 33612, USA.

*Jose D. Sandoval-Sus and Julio C. Chavez contribute equally to this paper.

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Introduction

With over 14,600 new cases reported in 2015, chronic lymphocytic leukemia (CLL) is the most common leukemia diagnosed in both the United States and western hemisphere [1,2]. Although CLL has classically been characterized as an 'indolent' B-cell malignancy, it has a heterogeneous clinical course and survival outcomes vary among different patients [2-4]. Staging systems focused on readily available clinical parameters have revealed a strong correlation between disease burden and overall survival (OS) (Rai and Binet staging) [5-7]. Both staging systems are still widely used in clinical practice and implemented in patient stratification for clinical trials. Nonetheless, clinical staging falls short of differentiating between rapidly evolving cases requiring earlier treatment and asymptomatic cases that remain quiescent for decades without active therapies [3,7]. Ways to analyze the mutational status of the immunoglobulin heavy-chain variable region (*IGHV*) and the availability of fluorescence *in situ* hybridization (FISH) to evaluate genomic alterations in CLL cells have improved the ability to prognosticate outcomes in this disease.

The presence of heavy-chain variable region mutations has a well-established prognostic role in CLL, with patients harboring a non-homologous *IGHV* sequence of >2% from the germline gene (mutated *IGHV*) having longer treatment-free intervals and better OS than their unmutated counterparts [8-11]. CLL patients with unmutated *IGHV* also have shorter disease control than CLL patients with mutated *IGHV* when treated with fludarabine, cyclophosphamide, and rituximab [12]. Lastly, unmutated *IGHV* CLL status has been correlated with higher prevalence of poor-risk features such as elevated ZAP-70 and CD38 and unfavorable mutations (i.e. del17p) [8,10,11]. More recently, the relevance of *IGHV* mutational status in CLL risk stratification was further bolstered when it was integrated in the CLL international prognostic score (CLL-IPI) [13]. A multivariate analysis of various established CLL prognostic factors, in 3472 treatment-naive CLL patients, identified five prognostic variables capable of strongly predicting five-year OS, with del17p/TP53 mutations, *IGHV* mutational status, and β_2-microglobulin (β_2M) having the highest impact. Importantly, patients with the *IGHV* 3-21 gene segment were the exception regarding the prognostic influence of *IGHV* status, with disease behavior similar to that shown with an unmutated clone, including higher prevalence of associated TP53 mutations and inferior survival [14-17].

Döhner et al. [18] used FISH to demonstrate that >80% of CLL patients had at least one genomic alteration at diagnosis. It was established that patients with sole del13q, trisomy 12, and normal FISH had a better OS (133, 114 and 111 months, respectively) than patients with del17p or del11q (OS of 32 and 79 months, respectively) [18]. Clinical information regarding CLL prognosis in patients with sole favorable genetic alterations and *IGHV* mutational status is scarce. In a retrospective analysis [4], 13 CLL patients with sole del13q mutation and unmutated *IGHV* had higher rates of progression, were more likely to receive CLL-directed therapies, and had inferior OS compared with patients with mutated *IGHV* and sole del13q. The prognostic impact of trisomy 12 as the single cytogenetic aberrancy in CLL patients, in relation to their *IGHV* mutational status, is uncertain. Although Döhner et al. [16] correlated this genetic aberration with a favorable treatment-free interval and prolonged survival, only 42% of patients in this genetic group harbored trisomy 12 as the

only genetic aberration. In an Italian retrospective analysis, 70% of patients harboring trisomy 12 mutations showed isolated trisomy 12 (through FISH at diagnosis) as the sole genetic abnormality [19]. Interestingly, mutations in the *NOTCH1* gene, which are associated with poor clinical outcomes in CLL [20,21], were significantly more prevalent in patients with isolated trisomy 12 than in those having trisomy 12 and other concomitant cytogenetics lesions (30% vs. 6%; p=.008) [19]. Also, *NOTCH1* mutations were associated with unmutated *IGHV* genes (84%; p=.003) [17].

At diagnosis, approximately 18% of CLL patients do not have identifiable cytogenetic abnormalities [18]. Similar to trisomy 12, this CLL population has been previously classified as having an intermediate clinical prognosis [18]; however, the influence of *IGHV* mutational status in their prognosis has not been evaluated. Here, our aim was to analyze the prognostic impact of *IGHV* mutational status in newly diagnosed CLL patients with del13q, trisomy 12, or negative FISH as sole genetic aberrations.

Materials and methods

Using the total cancer care (TCC) and Moffitt cancer center (MCC) malignant hematology databases, we retrospectively identified all patients with established CLL diagnosis from January 2000 to December 2013. Data included were CD38 and ZAP-70 expression, β_2M level (the upper limit of normal of β_2M at MCC was 2.2 mg/dL), conventional cytogenetics, interphase FISH and *IGHV* mutational status, and patient demographics. *IGHV* status was assessed at the patient's first evaluation at MCC. We included only patients with favorable genetic information (del13q, trisomy 12, and negative FISH) and *IGHV* mutational status. This study was approved by the Institutional Review Board of the University of South Florida. For this Health Insurance Portability and Accountability Act-compliant study, informed consent was not required, although patient consent was obtained prospectively prior to database inclusion.

FISH and IGHV analyses

Fluorescence *in situ* hybridization was done by interphase analysis utilizing probes for five centromeres: 11cen/11q22.3 (ATM), 12cen/12q15 (MDM2), 13q14 (D13S319)/13q34 (LAMP1), 17cen/17p13.1 (p53), and 11q13 (CCND1-XT)/14q32 (IGH-XT). *IGHV* mutational status was performed using cycle sequencing analysis. Mutated *IGHV* and unmutated *IGHV* were defined as non-homologous immunoglobulin sequence of ≥2% and <2% from the baseline sequence, respectively [9,22-24].

Statistical analyses

Patient demographic data were analyzed using descriptive statistics. Chi-square test was used to compare categorical variables between patient groups. Time-to-first treatment (TFT) was calculated as time from diagnosis to date of first prescribed antineoplastic therapy, and OS was calculated from diagnosis to the time of death or last recorded follow-up. Event time distributions were estimated by the Kaplan-Meier estimator and compared using log-rank test. Cox proportional hazard models were used to estimate hazard ratios and p values. We used SAS 9.4 software (Cary, NC) for statistical analyses.

Results

Demographics and clinical findings

Between 2000 and 2013, 1267 CLL patients were seen at our institution. Cytogenetic data were available for 601 (47%). Moreover, 338 individuals (26.5%) had *IGHV* mutational analyses performed and readily available. Of these, 273 CLL patients had both cytogenetic and *IGHV* mutational status information available: 145 patients (53.1%) had isolated del13q, 49 patients (17.9%) had isolated trisomy 12, and 79 patients (28.9%) had a negative FISH (Figure S1 in the Supplementary material). Patient demographics and clinical profile based on their IGHV status are summarized in Tables 1-3. Of 273 patients included in our study, 173 were men (63.3%) and 100 were women (36.6%) with overall median age at diagnosis of 59 years (34–83; 52% were younger than 60 years), which was similar to the median age for the whole cohort (60 years; 23–89 years). Long-term follow-up (median 7.8 years; 0.04–28.5) showed that more than half of survivors in our favorable genomic status patient group did not require CLL-directed treatment, underscoring the overall benign risk profile of this population.

In the isolated del13q (favorable cytogenetic risk) group, 49 patients (33.7%) harbored unmutated *IGHV* and 96 (66.2%) had mutated status. A larger proportion of patients with unmutated *IGHV* had ZAP-70 (42.8% vs. 13.5% with mutated; $p<.001$) and CD38 overexpression (24.4% vs. 10.4% with mutated; $p=.04$) and showed greater likelihood to require CLL-directed therapy (69.3% vs. 32.2% with mutated; $p\leq.001$). However, patients with mutated *IGHV* had a trend ($p=.06$) toward having a lower-risk disease by Rai staging (i.e. Rai stage 0–II; Table 1). In the isolated trisomy 12 cohort, 32 patients (65%) had unmutated and 17 patients (34.6%) had mutated *IGHV*, with no significant differences. In this cohort, more patients with unmutated *IGHV* genes had ZAP-70 overexpression at initial diagnosis, but both populations were otherwise similar (Table 2).

Regarding the negative FISH CLL cohort, 38 patients (48%) had unmutated and 41 patients (51.8%) had mutated *IGHV* status. Those with an unmutated *IGHV* status had a higher frequency of CLL-directed therapies (63.1%) than those with mutated *IGHV* status (36.5%; $p=.02$) and a proportionally higher trend (47.3%) of CD38 overexpression at baseline than those with unmutated status (29.2%), albeit not statistically significant ($p=.08$; Table 3). Median TFT and OS Kaplan-Meir curves for each FISH genetic category are noted in Figure S2.

IGHV status as a prognostic factor in CLL patients with isolated del13q

The median number of therapies among unmutated *IGHV* patients was 1 (0–4), with allogeneic hematopoietic cell transplantation (allo-HCT) used in four patients. Patients with mutated *IGHV* received 0.5 median treatments (0–4), with three patients requiring allo-HCT. The median TFT of patients harboring an isolated de13q was 6.4 years (95% CI, 4.51–13.06), with TFT being significantly shorter for patients with unmutated *IGHV* (2.98 years; 95% CI, 1.34–4.13) than with mutated *IGHV* genes (17.44 years; 95% CI, 6.86 to not reached; $p<.001$), with a hazard ratio (HR) of 3.41 (95% CI, 2.07–5.26, $p<.001$) (Figure 1 and Table S1 in the Supplementary material). In a preplanned multivariable analysis of

different baseline prognostic risk factors, unmutated *IGHV* status prevailed as an independent risk factor for shorter TFT in CLL patients (HR 10.7; 95% CI, 2.97–38.6; $p<.001$). Other risk factors that significantly correlated with earlier treatment were CD38 ($p=.003$) and ZAP-70 positive ($p=.024$) (Tables S2 and S3 in the Supplementary material).

In patients harboring sole del13q, the median OS for the whole group was 14.32 years (95% CI, 12.50 to not reached); a higher OS was shown in patients with mutated *IGHV*, with OS not reached at the time of this analysis (95% CI, 13.04 years to not reached), versus that shown in patients with unmutated *IGHV* (10.45 years; 95% CI, 9.10 to not reached; $p=.0026$) (Figure 1). A univariate analysis showed that unmutated *IGHV* status was strongly associated with worse survival in patients with isolated del13q (HR for unmutated *IGHV* status was 3.48; 95% CI, 1.469–8.240; $p=.0046$) (Table S1 in the Supplementary material). Nonetheless, when several baseline CLL prognostic risk factors were analyzed in a multivariate model, none of the variables correlated with OS. Of the patients who died in this group, 13 had unmutated *IGHV* and 9 had mutated *IGHV*, although none harbored *IGHV* 3–21 mutations [14,15]. All patients who died received at least one line of CLL-directed therapy, and none of them had Richter transformation. However, most patients had documented disease progression at time of death.

IGHV mutation as a prognostic factor for CLL with isolated trisomy 12 and negative FISH

We grouped CLL patients with either isolated trisomy 12 or negative FISH under the intermediate cytogenetic risk category based on the established prognostic role of these genetic abnormalities in this disease [7,18]. Among patients with sole trisomy 12, the median number of CLL-directed treatments was similar in both *IGHV* groups (median of 1; 0–3), and none with sole trisomy 12 underwent salvage allo-HCT or experienced Richter transformation. The median TFT for patients with sole trisomy 12 was 3.74 years (95% CI, 2.17–7.95). Of note, in this isolated abnormality group, there were no significant differences in TFT between those with unmutated (6.06 years; 95% CI, 1.88–7.70) versus those with mutated *IGHV* (3.74 years; 95% CI, 0.28 to not reached; $p=.551$), with a HR of 1.29 (95% CI, 0.56–2.95) for unmutated *IGHV* (Figure 2). In addition, median OS for patients with sole trisomy 12 was 12.78 years (95% CI, 11.50 years to not reached) without statistical differences between unmutated (median OS not reached) and mutated *IGHV* CLL (median OS of 12.78 years; $p=.28$). Univariate analysis did not show any significant differences in TFT or OS based on the *IGHV* mutational status (Table S1 in the Supplementary material). Nine patients in this cytogenetic category succumbed to their disease (five with unmutated and four with mutated *IGHV*, although none with *IGHV* 3–21 mutations) [14,15].

The median number of CLL treatments received by patients without identifiable cytogenetic abnormalities by FISH (isolated normal karyotype) was 1 (1–4 treatments). Within this group, four patients underwent allo-HCT (two with unmutated and two with mutated *IGHV*). Three of these patients were alive at the time of our data analyses. Of note, the patient who died after allo-HCT harbored unmutated *IGHV* and experienced clonal evolution by acquiring del17p mutation after the first administered chemoimmunotherapy. The median TFT of patients with negative FISH was 4.20 years (95% CI, 3.01–10.16 years), with shorter intervals in patients with unmutated (3.10 years, 95% CI, 2.37–4.42) versus

mutated *IGHV* (9.75 years, 95% CI, 3.27–21.26; p=.053). Univariate analysis showed HR of 1.909 (95% CI, 0.979–3.720) for unmutated *IGHV* (Figure 3 and Table S1 in the Supplementary material). Multivariate analysis showed that CD38 overexpression and low serum albumin levels at diagnosis (defined as ≤3.7 mg/dL) were independent risk factors for shorter TFT in this group; however, unmutated *IGHV* was not identified as an individual prognostic variable in this model (Tables S2 and S3 in the Supplementary material). The median OS for these patients had not been reached at the time of data analyses, although the 10-year OS of those with mutated *IGHV* (88%) versus unmutated *IGHV* (78%) in this genetic subgroup did not differ significantly (HR of 3.13, 95% CI, 0.631–15.5; p=.162). Of the nine patients who died in this group, two had mutated *IGHV*, although without *IGHV* 3–21 [14,15]. Two patients never received CLL treatment before they died (one from stage IV non-small cell lung carcinoma and one from unknown reasons). Also, none of the patients in this group had documented Richter transformation.

Discussion

During the past decades, the prognostication of CLL has evolved, especially with the integration of traditional prognostic markers with modern technologies (i.e. genomics) [3,5,6,9,18,24,25]. CLL cytogenetics, classically assessed by FISH and karyotyping, and *IGHV* mutational status determined by PCR are probably the most powerful and validated clinical prognostic biomarkers used in our daily practice [2,3,18,23]. CLL patients with unmutated *IGHV* usually have concomitant cytogenetic aberrations that have been strongly associated with dismal CLL-related outcomes, such as del11q and del17p [18,23,26]. On the other hand, del13q has classically been correlated with mutated *IGHV* [4,23,26].

As previously noted, reports are scarce on the prognostic relation between isolated good (del13q) and intermediate-risk cytogenetic aberrations (trisomy 12 or negative FISH) and the mutational changes of the *IGHV* homology sequence in CLL. To our knowledge, only one retrospective study has explored the prognostic relevance of *IGHV* mutational changes in CLL with sole del13q. Gladstone et al. retrospectively identified 47 CLL patients with concomitant isolated del13q and available *IGHV* mutational status (34 with mutated and 13 with unmutated *IGHV*) [4]. Our report, which comprised three times as many patients with the above characteristics, demonstrated prevalence of unmutated *IGHV* of 33% (49/145), which is slightly higher than previously reported [4,26]. Importantly, although this cohort of patients had a fairly indolent disease course, those with unmutated *IGHV* were more likely to receive treatment for CLL and treatment was initiated significantly earlier, as previously reported [4]. The most clinically relevant association observed in this population was the dismal prognosis that unmutated *IGHV* confers on patients with isolated del13q. Indeed, 8 of 10 CLL patients with this mutational aberration harboring a mutated *IGHV* gene were alive 10 years after diagnosis, whereas more than half of those with unmutated *IGHV* had died. Although our study had a longer follow-up, our findings are in accordance with those previously observed [4]. Nonetheless, we could only identify *IGHV* as an independent prognostic factor for TFT but not for OS. This is in line with findings reported by other groups that found that *IGHV* mutational status in CLL patients also failed to independently prognosticate OS [4]. Although elevated CD38 overexpression and advanced Rai stage at diagnosis were strongly associated with a shorter TFT, no other baseline variable

independently predicted mortality in patients harboring del13q. As such, patients with del13q included in our study were younger at diagnosis than usual; albeit not significantly, with the proportion of unmutated *IGHV* patients with isolated del13q who were <60 years old being slightly higher. This observation, which was also noted in a previous retrospective analysis, may be explained by referral bias [4,26]. At least one large report described a significantly higher incidence of unmutated *IGHV* in CLL patients 55 years or younger who also had a worse OS compared with a normal population group matched by sex and age [1,4,27,28]. These observations are pertinent because, despite those with isolated del13q CLL carrying a better clinical outlook as a whole, younger patients may be prone to having more genotoxic treatments in the pursuit of long-lasting remission [29,30]. In turn, these patients could develop a molecularly unstable disease, thus perhaps generating high-risk CLL clones linked to dismal outcomes [31-33].

Although controversial, CLL patients with sole trisomy 12 or without identifiable cytogenetic changes by FISH are classified under an intermediate-risk group based on the seminal work of Döhner et al., which was reiterated in a more contemporary analysis by Rossi et al. [18,34]. The prognostic influence of the *IGHV* mutational status in these specific cytogenetic risk subgroups has not been previously reported. While the sole trisomy 12 group was acknowledged as the smallest one in sample size, the prevalence of this isolated mutation (18%) was similar to that reported in previous larger studies of newly diagnosed CLL patients [35]. Although patients with unmutated *IGHV* phenotype expressing higher ZAP-70 levels at diagnosis were slightly more prevalent in this cohort, there were no significant differences among the groups. Nonetheless, patients with unmutated or mutated *IGHV* genes harboring trisomy 12 as the only CLL-related genetic aberration showed a fairly indolent disease, with OS in excess of 10 years.

It is possible that outcomes of this CLL population might be dictated by more complex biologic factors other than *IGHV* mutational status. The prognosis of trisomy 12 CLL may also be influenced by its allele the burden. It seems that worse outcomes are seen if more than 60% CLL cells harbor trisomy 12, especially in the presence of 11q deletion [36]. For instance, *NOTCH1* mutations occur in approximately 30% of CLL patients harboring an isolated trisomy 12 [19]. Mutations in the PEST domain of the key ligand-activated transcription factor of the *NOTCH* signaling pathway, *NOTCH1*, were among the first novel molecular aberrations discovered by next-generation sequencing (NGS) in CLL [25,37], and it correlates with early disease progression, rituximab-based therapy refractoriness, Richter transformation, and shorter survival [21,37-40].

Patients clustered on the other arm of the intermediate-risk subgroup had no identifiable CLL-related cytogenetic aberrations. Interestingly, they had the longest survival of the three groups. Moreover the *IGHV* mutational profile had no prognostic influence on survival of these patients. Conversely, patients with negative FISH CLL harboring unmutated *IGHV* genes tended to have earlier disease progression and were more likely to require specific CLL treatment faster. Using FISH, which is currently recommended by several CLL clinical guidelines to assess baseline cytogenetics [2,3,41,42], approximately 20% of newly diagnosed patients lack mutational aberrations [18]. Apparently, the prognosis of these patients is probably determined by other molecular signatures. Thus, Jeromin et al. [43]

analyzed a large cohort of untreated CLL patients for several genetic markers (including *SF3B1, NOTCH1, FBXW7, MYD88*) using direct Sanger sequencing and NGS. In patients with negative FISH only, mutations in the spliceosome protein *SF3B1* were frequent (15% of patients), whereas *NOTCH1* mutations rarely occurred. In multivariate analyses both *SF3B1* mutations and *IGHV* mutational status had independent prognostic impact in TFT and OS. Further investigations into the prognostic influence of *IGHV* mutational status in CLL patients carrying novel described mutations (i.e. *BIRC3*) may help in understanding the prognosis of this patient subgroup.

Our report has several limitations. First, this was a single center retrospective study, and our results may be affected by an inherent referral bias. However, most patients had an overall low-risk disease profile and were not heavily pretreated before referral. Also, their community oncologists consistently followed them throughout the study period, including having occasional visits to our center. Therefore, our results may more accurately portray 'real-world' disease progression, reaffirming the biologic relevant relation between *IGHV* and these cytogenetic groups, especially for del13q. Also, the relatively small sample size of certain cytogenetic risk groups (i.e. isolated trisomy 12), and thus low incidence of the measured outcomes, may have underestimated some of the studied endpoints. Although missing information is an inherent deficiency of large retrospective cohorts where data from multiple sources is reviewed, it was also quite informative because it gave us a scope of the current prognostic stratification practices in CLL performed both in the community at large and at our own institution. This is much more relevant at the present time with the advent of the CLL-IPI score, which accentuates the prognostic relevance of both *IGHV* mutational status and β_2M level [13].

In conclusion, our study further expands the evidence suggesting that CLL with isolated good cytogenetic aberrations (del13q) and unmutated *IGHV* could represent a different disease subgroup characterized by worse biologic characteristics and more dreadful clinical outcomes. Although we could only detect a possible association between *IGHV* mutations and the need for earlier treatment in patients with negative FISH, the biologic effects of the *IGHV* status did not alter survival outcomes in patients within the intermediate cytogenetic group. Backed by a substantial amount of evidence confirming the prognostic importance of *IGHV* [13,29,30,44] and the usefulness of β_2M [13,45], we propose that both tests should be included in the initial diagnostic work-up of all CLL patients, along with the already recommended FISH-based cytogenetic analysis [13]. The routine use of this prognostic work-up in newly diagnosed CLL patients should be widely expanded to the community, and its cost-effectiveness should be studied in real-world settings. Also, further research on *IGHV* mutational status distribution and on its prognostic impact in CLL patients with well-established poor prognostic recurrent mutations (including, *NOTCH1, TP53, SF3B1, ATM, BIRC3*), as detected through NGS, is needed [25]. Expanding this molecular knowledge might be especially relevant in patients with either isolated trisomy 12 or normal cytogenetics. These studies could potentially be carried though multi-institutional collaborations, utilizing prospective comprehensive prognostic information from phase 3 clinical studies and/or robust single center databases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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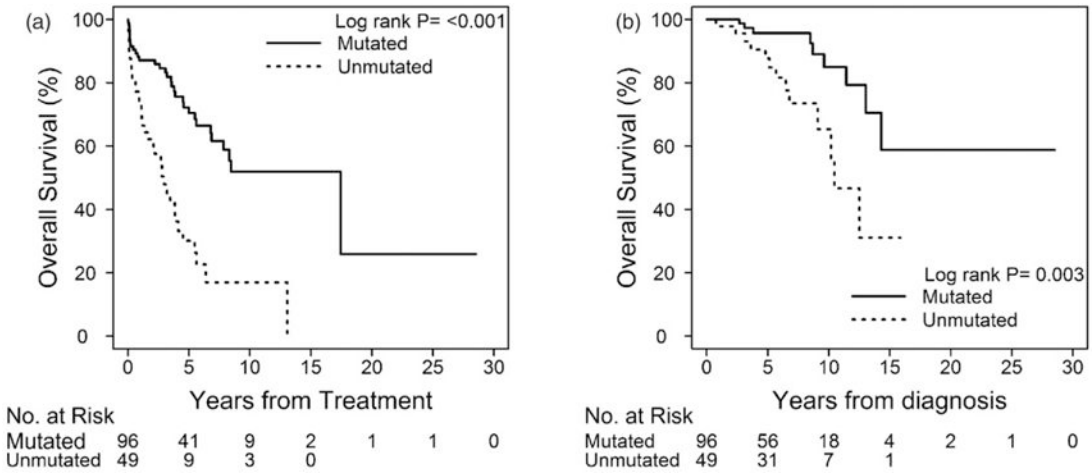

Figure 1.

Time to first treatment and overall survival of patients with isolated del13q based on *IGVH* mutational status.

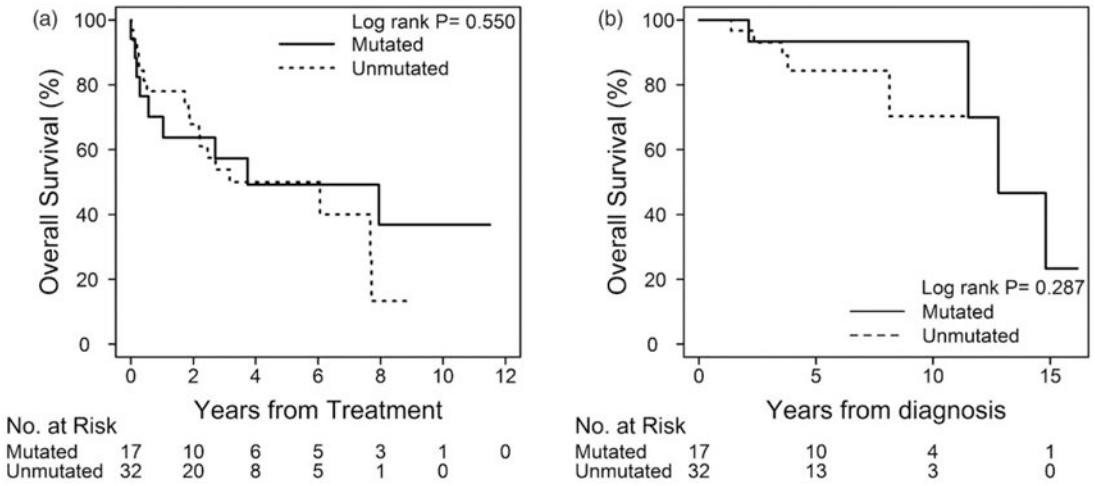

Figure 2.

Time to first treatment and overall survival of patients with isolated trisomy 12q based on *IGVH* mutational status.

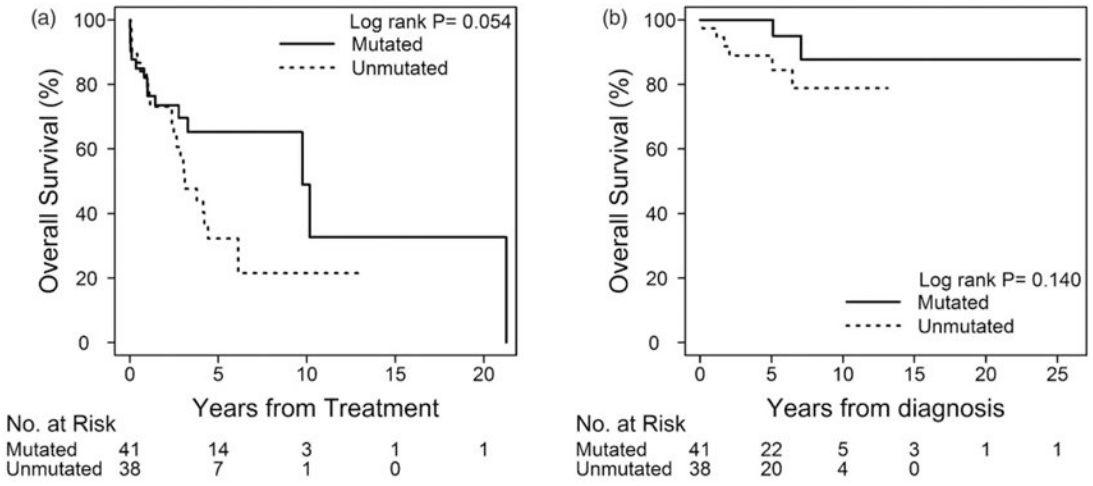

Figure 3.

Time to first treatment and overall survival of patients with normal karyotype based on *IGVH* mutational status.

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Table 1.

Patient characteristics with isolated del13q (overall and per *IGVH* status).

	Del13q			
	Overall (*n*=145)	***IGHV*<2% (*n*=49)**	***IGHV*≥2% (*n*=96)**	***p* Value***
Age at diagnosis, median (range), years	59 (36–83)	58 (37–76)	60 (36–83)	
Age				
Age >60 years	71 (49.0%)	23 (47.0%)	48 (50.0%)	.86
Age <60 years	74 (51.0%)	26 (53.1%)	48 (50.0%)	
Sex				
Male	91 (62.8%)	30 (61.2%)	61 (63.5%)	.85
Female	54 (37.2%)	19 (38.7%)	35 (36.4%)	
Modified Rai staging				
Rai 0–II	109 (75.1%)	30 (61.2%)	79 (82.2%)	.063
Rai III–IV	27 (18.6%)	13 (26.5%)	14 (14.5%)	
Unknown	9 (6.2%)	8 (16.3%)	12 (12.5%)	
ZAP-70				
>20%	34 (23.4%)	21 (42.8%)	13 (13.5%)	<.001
<20%	70 (48.2%)	17 (34.6%)	53 (55.2%)	
Unknown	41 (28.2%)	11 (22.4%)	30 (31.2%)	
CD38				
>30%	22 (15.1%)	12 (24.4%)	10 (10.4%)	.043
<30%	92 (63.4%)	27 (55.1%)	65 (67.7%)	
Unknown	31 (21.3%)	10 (20.4%)	21 (21.8%)	
β2M				
Elevated	36 (24.8%)	14 (28.5%)	22 (22.9%)	.362
Not elevated	52 (35.8%)	15 (30.6%)	37 (38.5%)	
Unknown	57 (39.3%)	20 (40.8%)	37 (38.5%)	
CLL treatment				
No	80 (55.2%)	15 (30.6%)	65 (67.7%)	<.001
Yes	65 (44.8%)	34 (69.3%)	31 (32.2%)	
Unknown	0 (0.0%)	0 (0.0%)	0 (0.0%)	

β2M: β2-microglobulin; CLL: chronic lymphocytic leukemia; IGVH: immunoglobulin variable heavy chain.

* A $p<.05$ was considered a statistical difference.

Table 2.

Patient characteristics with isolated trisomy 12 (overall and per *IGVH* status).

	Trisomy 12			
	Overall (*n*=49)	***IGHV*<2% (*n*=32)**	***IGHV*≥2% (*n*=17)**	***p* Value***
Age at diagnosis, median (range), years	57 34–74	58 34–74	55 34–71	
Age				
Age >60 years	31 (39.2%)	17 (44.7%)	14 (34.1%	.365
Age <60 years	48 (60.7%)	21 (55.2%)	27 (65.8%)	
Gender				
Male	51 (64.5%)	25 (65.7%)	26 (63.4%)	1
Female	28 (35.4%)	13 (34.2%)	15 (36.5%)	
Modified Rai staging				
Rai 0–II	63 (78.4%)	29 (76.3%)	34 (82.9%)	.72
Rai III–IV	9 (10.1%)	5 (13.1%)	4 (9.7%)	
Unknown	7 (11.4%)	4 (10.5%)	3 (7.3%)	
ZAP-70				
>20%	23 (27.8%)	12 (31.5%)	11 (26.8%)	.44
<20%	41 (41.7%)	17 (44.7%)	24 (58.5%)	
Unknown	16 (30.3%)	9 (23.6%)	6 (14.6%)	
CD38				
>30%	20 (25.3%)	18 (47.3%)	12 (29.2%)	.08
<30%	37 (46.8%)	14 (36.8%)	23 (56.0%)	
Unknown	12 (15.1%)	6 (15.7%)	6 (14.6%)	
β2M				
Elevated	16 (20.2%)	6 (15.7%)	10 (24.3%)	.54
Not elevated	31 (39.2%)	15 (39.4%)	16 (39.0%)	
Unknown	32 (40.5%)	17 (44.7%)	15 (36.5%)	
CLL treatment				
No	40 (50.6%)	14 (36.8%)	26 (63.4%)	.02
Yes	39 (49.3%)	24 (63.1%)	15 (36.5%)	
Unknown	0 (0.0%)	0 (0.0%)	0 (0.0%)	

β2M: β2-microglobulin; CLL: chronic lymphocytic leukemia; *IGVH*: immunoglobulin variable heavy chain.

* $p < .05$.

Table 3.

Patient characteristics with normal karyotype; overall and per *IGVH* status.

	Normal karyotype			
	Overall (*n*=79)	***IGHV*<2% (*n*=38)**	***IGHV*≥2% (*n*=41)**	***p* Value***
Age at diagnosis, median (range), years	57 34–74	58 34–74	55 34–71	
Age				
Age >60 years	31 (39.2%)	17 (44.7%)	14 (34.1%	.365
Age <60 years	48 (60.7%)	21 (55.2%)	27 (65.8%)	
Gender				
Male	51 (64.5%)	25 (65.7%)	26 (63.4%)	1
Female	28 (35.4%)	13 (34.2%)	15 (36.5%)	
Modified Rai staging				
Rai 0–II	63 (78.4%)	29 (76.3%)	34 (82.9%)	.72
Rai III–IV	9 (10.1%)	5 (13.1%)	4 (9.7%)	
Unknown	7 (11.4%)	4 (10.5%)	3 (7.3%)	
ZAP-70				
>20%	23 (27.8%)	12 (31.5%)	11 (26.8%)	.44
<20%	41 (41.7%)	17 (44.7%)	24 (58.5%)	
Unknown	16 (30.3%)	9 (23.6%)	6 (14.6%)	
CD38				
>30%	20 (25.3%)	18 (47.3%)	12 (29.2%)	.08
<30%	37 (46.8%)	14 (36.8%)	23 (56.0%)	
Unknown	12 (15.1%)	6 (15.7%)	6 (14.6%)	
β2M				
Elevated	16 (20.2%)	6 (15.7%)	10 (24.3%)	.54
Not elevated	31 (39.2%)	15 (39.4%)	16 (39.0%)	
Unknown	32 (40.5%)	17 (44.7%)	15 (36.5%)	
CLL treatment				
No	40 (50.6%)	14 (36.8%)	26 (63.4%)	.02
Yes	39 (49.3%)	24 (63.1%)	15 (36.5%)	
Unknown	0 (0.0%)	0 (0.0%)	0 (0.0%)	

β2M: β2-microglobulin; CLL: chronic lymphocytic leukemia; *IGVH*: immunoglobulin variable heavy chain.

* $p<.05$.