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Authors
Strati, Paolo
Abruzzo, Lynne V
Wierda, William G
et al.

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Second Cancers and Richter's Transformation are the Leading Causes of Death in Patients with Trisomy 12 Chronic Lymphocytic Leukemia

Paolo Strati, MD[1], **Lynne V. Abruzzo, MD, PhD**[2], **William G. Wierda, MD, PhD**[1], **Susan O'Brien, MD**[1], **Alessandra Ferrajoli, MD**[1], and **Michael J. Keating, MD**[1]

[1]Department of Leukemia, The University of Texas M. D. Anderson Cancer Center, Houston, Texas, 77030 USA

[2]Department of Hematopathology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas, 77030 USA

Abstract

Trisomy 12 (+12) is detected by fluorescence *in situ* hybridization (FISH) analysis in up to 20% of patients with chronic lymphocytic leukemia (CLL). Patients with +12 are known to have unique features and to carry an intermediate prognosis. In order to better define this large group, we reviewed the characteristics of 250 untreated patients with +12. When compared to 516 untreated patients negative for +12 by FISH, patients with +12 showed a higher incidence of thrombocytopenia, Richter Transformation (RT) and second malignant neoplasms (SMN), in addition to the expected increased rate of CD38 positivity and atypical immunophenotype. At a median follow-up of 51 months, 57% of patients needed first-line treatment; median time-to-first-treatment was 38 months and on multivariate analysis (MVA) it was shorter in patients with advanced Rai stage, palpable splenomegaly, and deletion 14q by conventional cytogenetic analysis. The overall response rate with first-line treatment was 94%. The median failure-free survival has not been reached, but on MVA it was shorter in patients who achieved a response other than complete remission or with FISH negativity for deletion 13q. The median overall survival for the entire group has not been reached, but on MVA it was shorter in patients with an absolute lymphocyte count >30×10^9/L or who developed SMN. Eighteen deaths have been observed so far, and RT and SMN were the leading causes of death (3 and 6, respectively). In

Contact information for correspondence: Michael J. Keating, MBBS, Department of Leukemia, The University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Blvd., Unit 428, Houston, TX 77030, USA, ; Email: mkeating@mdanderson.org, Telephone: +1 (713) 745-2376, Fax: +1 (713) 794-1602.

Authorship Contributions

M.J.K. and P.S. designed the study, analyzed the data, and wrote the paper; A.F. designed the study and provided supervision and advice in data analysis and manuscript preparation; L.V.A. supervised the conventional cytogenetic and FISH testing and provided advice and oversight in manuscript preparation; W.W. and S.O. contributed to the design of the study, verified the accuracy of patient data, and provided advice on statistical analysis.

Disclosure of Conflict of Interest

The authors declare no competing financial interests.

conclusion, patients with +12 CLL show characteristic clinical and biological features, and may benefit from increased surveillance for second cancers.

Keywords

CLL; trisomy 12; prognosis; second cancers; Richter transformation

Introduction

Chronic lymphocytic leukemia (CLL) is a B-cell malignancy with a variable clinical course for which clonal genomic aberrations play a crucial prognostic role. Trisomy 12 (+12) is the most common abnormality identified by chromosome banding analysis (CBA). It is the third most common abnormality identified by fluorescence *in situ* hybridization (FISH) analysis using a panel of FISH probes to the common recurrent abnormalities (deletions of 11q, 13q, 17p, and +12), with an incidence of about 16%.[1] Trisomy 12 is traditionally associated with an intermediate risk of progression, and a favorable overall survival (OS).[2] CLL cells with +12 tend to have atypical morphology, defined as more than 15% of cells with cleaved nuclei and/or lymphoplasmacytoid features, and an atypical immunophenotype, with a modified Matutes score less than 4 (based on expression of CD5, CD23, FMC7, surface immunoglobulin, CD22 and/or CD79b).[3–6]

In CLL cases with +12 identified by FISH, it is the sole aberration in about 70% of cases. It is associated with deletion 13q (del(13q)), deletion 11q (del(11q)), and deletion 17q (del(17p)) in 18%, 8%, and 4% of cases, respectively.[7] By CBA, +12 is identified as the sole abnormality in 30% of cases, but it may also be associated with trisomy 18 (+18, 5% of cases), deletion 14q (del(14q), 3% of cases), t(14;19)(q32;q13), and/or trisomy 19 (+19).[1,8,9] Of interest, the incidence of +12 rises from 16 to 36% in cases of small lymphocytic lymphoma (SLL)[10], and small case series have reported an incidence of +12 in up to 50–90% of patients with Richter's Transformation (RT)[11–13], though the mechanism remains unclear.[14]

Recently, interest in +12 CLL has been raised by the discovery of *NOTCH1* mutation in up to 24% of CLL patients with +12, particularly in cases with somatically unmutated immunoglobulin heavy chain variable region (*IGHV)* genes. *NOTCH1* mutation is a stable marker, and may be associated with an inferior outcome.[7,15,16] However, the impact of *NOTCH1* mutation on prognosis may be influenced by other concurrent chromosomal aberrations. For example, *NOTCH1* mutation occurs more frequently in patients carrying +12 as the sole abnormality, but a worse outcome is observed among patients with +12 associated with additional chromosomal abnormalities, irrespective of *NOTCH1* mutation status.[17]

Compared to other cytogenetic subtypes, there are few large series in the literature that describe the clinical features of CLL cases with +12;[18–21] the largest includes 104 patients.[7] Thus, we analyzed and summarized our single-center experience of the clinical and laboratory features of 250 previously-untreated patients with +12 CLL over a period of nine years. Similar to previous reports, we observed an association between +12 and atypical

immunophenotype, and a worse outcome in presence of deletion 14q (del14q). However, in contrast to previous reports, we observed an elevated mortality related to the onset of second cancers, suggesting the need for increased surveillance in this genetic subgroup.

Methods

Case selection

We performed a retrospective analysis of 250 treatment-naïve patients with CLL and +12 seen and followed at the University of Texas M.D. Anderson Cancer Center (MDACC) between 2003 (when routine FISH analysis was implemented at MDACC) and 2011. Their baseline characteristics were compared to those of 516 treatment-naïve patients with CLL and negative FISH followed at MDACC in the same time period. The study was approved by and conducted according to the Institutional Review Board of MDACC guidelines and was conducted in accordance with the principles of the Declaration of Helsinki. The clinical and laboratory features were obtained by review of the medical records. Cases were classified using the hierarchical risk model of FISH anomalies.[2] Thus, we included cases with del(13q) or diploid cytogenetics in our analysis, but excluded cases with del(11q) or del(17p). The National Cancer Institute-Working Group (NCI-WG) criteria were applied to initiate treatment and to categorize response to treatment and time-to-event endpoints.[22,23] We classified front-line therapy as follows: (1) FCR-based regimens, which included fludarabine, cyclophosphamide, and rituximab (FCR), FCR plus mitoxantrone (FCMR), FCR plus granulocyte-macrophage colony-stimulating factor (GM-CSF), FCR plus alemtuzumab (CFAR); (2) rituximab-based regimens, which included rituximab plus high-dose methylprednisone (HDMP), and rituximab plus GM-CSF; (3) investigational drug regimens, which included lenalidomide, idelalisib, and ibrutinib, with or without rituximab.

Routine laboratory and cytogenetic analyses

Laboratory testing, including evaluation of the *IGHV* somatic mutation status, and expression of CD38 and ZAP70, were performed as described previously.[24,25] CBA was performed on metaphase cells prepared from bone marrow aspirate specimens cultured for 24 hours without mitogens, or for 72 hours with lipopolysaccharide, using standard techniques. Twenty Giemsa-banded metaphases were analyzed, and the results were reported using the International System for Human Cytogenetic Nomenclature. FISH analysis was performed on interphase nuclei prepared from bone marrow samples after culturing cells for 24 hours without mitogens, using the CLL probe panel (Vysis) according to the manufacturer's recommendations. The panel includes probes specific to *TP53* (17p13.1), *ATM* (11q22.3), *D13S319* (13q14.3), *LAMP1* (13q34), and the centromeric region of chromosome 12 (12p11.1-q11). Results for 200 analyzed nuclei were reported.

Statistical analysis

Time-to-first-treatment (TTT) and overall survival (OS) were calculated from the date of diagnosis to the date of therapy and death, respectively, or the date of last follow-up. Failure-free survival (FFS) was calculated from the date of first therapy to the date of relapse, death, or last follow-up. Survival distributions were calculated using the method of Kaplan and Meier and were compared using the log-rank test. Categorical and continuous variables were

compared using the χ^2 or Fisher exact tests, or the Mann-Whitney test, as appropriate. Linear regression and Cox regression were used for multivariable analysis (MVA) of categorical variables and survival, respectively. All *P* values were two-sided and considered significant if ≤0.05.

Results

Patient characteristics

Two-hundred and fifty treatment-naïve CLL patients with +12 evaluated at MDACC between 2003 and 2011 were included in the study. Of the 250 patients, 191 were evaluated by both CBA and FISH, and 59 by FISH only. Of the 191 patients assessed by both FISH and CBA, 91 were FISH+/CBA+ and 100 were FISH+/CBA− for +12. Trisomy 12 was the only abnormality in 82% of cases by FISH and 21% of cases by CBA (Table I). Baseline patient characteristics at presentation to MDACC are shown in Table II. We compared these patients to a cohort of 516 treatment-naïve CLL patients who lacked the common recurrent abnormalities on FISH analysis (negative for deletions of 11q, 13q, 17p, or +12) evaluated at our institution during the same time period. On univariate analysis (UVA), factors associated with +12 were thrombocytopenia (platelets (PLT) $<100\times10^9$/L; p <0.001), bone marrow lymphocytosis (BM-L) ≥80% (p=0.05), serum beta-2-microglobulin (B2M) ≥4 mg/L (p=0.02), unmutated *IGHV* (p=0.02), positivity for CD38 (p<0.001), and Matutes score <4 (p<0.001). Factors not associated with +12 (p>0.05) were male gender, age ≥65years, bulky lymphadenopathy (LN), palpable splenomegaly, palpable hepatomegaly, hemoglobin (HGB) <10 g/dL, absolute lymphocyte count (ALC) $>30\times10^9$/L, positivity for ZAP70, and use of VH 4-34 or VH 3-23. On multivariate analysis (MVA), factors significantly associated with +12 were PLT $<100\times10^9$/L (odds ratio (OR) 2.4, p=0.03), positivity for CD38 (OR 2.4, p=0.001), and Matutes score <4 (OR 2.4, p <0.001) (Table II).

Time-To-first-Treatment

With a median follow-up of 51 months (range, 1–105), 142 patients (57%) required front-line therapy. The median TTT was 38 months (95% confidence interval [CI], 27–48), significantly shorter than FISH-negative patients (38 vs 82 months, p<0.001) (Figure 1A). On UVA, factors significantly associated with shorter TTT were Rai stage III–IV (p<0.001), bulky LN (p=0.02), palpable splenomegaly (p<0.001), HGB <11 g/dL (p<0.001), PLT $<100\times10^9$/L (p<0.001), ALC $\geq30\times10^9$/L (p<0.001), BM-L ≥80% (p=0.005), B2M ≥4 mg/L (p<0.001), positivity for ZAP70 (p=0.03), >50% of interphase nuclei positive for +12 by FISH (p=0.04), CBA positivity for +12 (p<0.001), aneuploidy on CBA (p<0.001), and CBA positivity for del(14q) (p=0.001). Factors not associated with shorter TTT on UVA (p>0.05) were male sex, age ≥65 years, palpable hepatomegaly, unmutated *IGHV*, positivity for CD38, use of VH4-34 or VH3-23, Matutes score <4, FISH positivity for del(13q), CBA positivity for +12 only, for +19, and for +18. The multivariable model for TTT included Rai stage III–IV (hazard ratio [HR] 3.3, p=0.02), palpable splenomegaly (HR 2.3, p=0.007), and CBA positivity for del(14q) (HR 3.5, p=0.004) (Figure 1B) as independently associated with TTT (Table III).

Response to First-Line Treatment and Failure-Free Survival

Front-line treatment consisted of an FCR-based regimen for 91 (64%) patients, an R-based regimen for 29 (20%), and investigational drug regimens for 22 (16%). A similar distribution of treatment regimens was observed in the control cohort. The complete remission (CR) rate was 52% (72%, 3%, and 3%, respectively); the overall response rate (ORR) was 94% (96%, 86% and 95%, respectively). With a median follow-up of 22 months (range, 1–85), the median failure-free-survival (FFS) following front-line treatment has not been reached (Figure 2A); 41 patients (29%) failed first therapy. On UVA, factors significantly associated with shorter FFS following first-line therapy were FISH negativity for del(13q) (p=0.003), therapy other than FCR-based (p<0.001), and response other than CR (p<0.001). Factors not significantly associated with FFS (p>0.05) were male sex, age >65 years, Rai stage III–IV, bulky LN, palpable splenomegaly or hepatomegaly, HGB <11 g/dL, PLT <100 $X10^9$/L, ALC ≥30×10^9/L, BM-L ≥80%, B2M ≥4 mg/L, unmutated *IGHV*, positivity for CD38 or ZAP70, use of VH4-34 or VH3-23, Matutes score <4, >50% of interphase nuclei positive for +12 by FISH, CBA positive for +12, aneuploidy on CBA, CBA positive for +12 only, CBA positive for +19, del14q or +18, achievement of PR. The multivariable model included FISH negativity for del(13q) (HR 6.2, p=0.01) (Figure 2B) and response other than CR (HR 3.3, p=0.003) (Figure 2C) as independently associated with FFS (Table III). Among the 41 patients who failed first-line therapy, 18 were observed (and 8 of them died), whereas 23 received 2nd line treatment (and 2 of them died). Second line treatment consisted of FCR-based regimen in 11 patients, R-based in 5, investigational in 4, and bendamustine plus rituximab in 3 patients. ORR was 83%.

Overall survival and causes of death

During the time of observation, the overall estimated median survival was not reached and it did not differ significantly between patients with or without +12 by FISH (p=0.22)(Figure 3A). On UVA, factors significantly associated with a shorter OS were age ≥65 years (p=0.02), HGB <11 g/dL (p=0.01), ALC ≥30 $X10^9$/L (p<0.001), B2M ≥4 mg/L (p=0.004), use of VH3-23 (p=0.05), aneuploidy on CBA (p=0.03), failure to achieve CR (p=0.03), onset of RS (p<0.001), and development of a second malignant neoplasm (SMN)(p<0.001). Factors not associated with OS on UVA (p>0.05) were male sex, Rai stage III–IV, bulky LN, palpable splenomegaly or hepatomegaly, PLT <100×10^9/L, BM-L ≥80%, unmutated *IGHV*, positivity for CD38 or ZAP70, use of VH4-34, Matutes score <4, positivity for del(13q) by FISH, >50% of interphase nuclei positive for +12 by FISH, CBA positivity for +12, CBA positivity for +12 only, +19, del14q, or +18, use of FCR-based therapy, and achievement of PR. In the multivariable model, ALC >300×10^9/L (HR 14.5, p=0.04) (Figure 3B) and development of a SMN (HR 23.8, p=0.002) (Figure 3C) remained independently associated with OS (Table III).

Overall, 6 patients (2%) developed histologically-confirmed RT after a median time of 10 months (95% CI, 5–15) from diagnosis. The incidence of RT was significantly higher among treated than untreated patients (4% vs 0%, p= 0.04); it was also higher among patients with +12 FISH-positive than in the control cohort (2 vs 0.4%, p=0.02). Twenty-two patients (9%) developed a SMN other than RT, after a median of 19 months (95% CI, 6–32) from diagnosis. Five SMN were hematologic malignancies (1 acute myeloid leukemia, 1

myelodysplastic syndrome, 1 myeloproliferative neoplasm, and 2 plasma cell myelomas) and 17 were non-hematologic malignancies (1 bladder carcinoma, 1 uterine carcinoma, 1 gastric carcinoma, 1 colon carcinoma, 1 lung carcinoma, 4 prostate carcinomas, 6 invasive squamous cell carcinomas of the skin, 1 sarcoma, 1 thyroid carcinoma). Of the 5 hematologic SMN, 2 occurred after therapy for CLL (after 18 from CFAR and 36 months from R+GMCSF) and, therefore, could be classified as therapy-related. The incidence of SMN did not differ significantly among treated and untreated patients (p=0.12), but it was significantly higher in +12 FISH-positive patients than in the control cohort (9% vs 1%, p<0.001).

Eighteen patients (7%) died during the time of observation, 15 of whom had received treatment for CLL. Seven patients died of CLL-related (4 patients) or RT-related (3 patients) causes, i.e. severe infections, bleeding, organ infiltration. Six patients died of a SMN (1 acute myeloid leukemia, 1 gastric carcinoma, 1 colon carcinoma, 1 lung carcinoma, 1 bladder carcinoma, 1 sarcoma), and 5 died of unrelated causes. The distribution of the causes of death did not differ significantly among treated and untreated patients (p=0.24).

Discussion

Although the most common cytogenetic abnormalities identified by FISH in CLL are deletions, +12 is the most commonly identified abnormality on conventional karyotypic analysis. Trisomy 12 has been traditionally considered an intermediate-risk prognostic factor in CLL.[2] Cases of CLL with +12 comprise up to 16% of all patients assessed by FISH, yet relatively little is known about its pathophysiology compared to other genetic subtypes. In the current study we analyzed 250 treatment-naive patients with +12 CLL, the largest series in the literature.

In our series, +12 was the sole abnormality in 82% of cases assessed by FISH; it was associated with del(13q) in the remaining cases, similar to previous reports.[7,18] Of interest, because we used the hierarchical FISH model, we excluded cases with associated del(11q) or del(17p) from our analysis.[2] When evaluated by CBA, +12 was the sole abnormality in 21% of cases; the remaining cases were diploid or showed +12 in association with +19, del14q, and +18, or less commonly, with trisomy 8, del(13q), and t(14;19)(q32;q13).

Recent data suggest that +12 associated with additional chromosomal abnormalities portends a poor prognosis, independent of *NOTCH1* mutation, which is more common in cases with isolated +12 compared to cases with additional abnormalities.[17] New methods to stimulate CLL cells to divide in culture (developed after the period of our current study) have increased the sensitivity to detect chromosomal abnormalities and karyotypic complexity.[26] Using these methods, it is unclear whether in cases with +12 karyotypic complexity or *NOTCH1* mutation status will have a greater impact on prognosis.

In our study, several clinical and laboratory features distinguished CLL patients with +12 from the cohort with negative FISH. Similar to previous studies, we found an association between +12, atypical immunophenotype (low Matutes score), and positivity for CD38 (that did not correlate with the somatic mutation status of the *IGHV* genes or positivity for

ZAP70).[3–5,20,21,27,28] CD38, whose expression is often associated with +12, is a highly conserved transmembrane glycoprotein that plays a critical role in lymphocyte trafficking between blood and lymphoid organs, and in survival and proliferation within the lymphoid organs. It is conceivable that CD38 inhibitors currently under development may have particular efficacy in this cytogenetic subtype.[29–32]

Unlike other studies, we found that patients with +12 were more likely to present with thrombocytopenia. During the course of disease, cytopenias have been observed in up to 24% of patients with CLL, resulting from bone marrow failure or autoimmune disease.[33,34] While the diagnosis of autoimmune hemolytic anemia is based on specific laboratory findings, identifying an autoimmune thrombocytopenia can be problematic.[35,36] In our series, bone marrow infiltration was not associated with +12 on MVA, suggesting an autoimmune etiology for the elevated rate of thrombocytopenia that we observed. A previous study showed that patients with +12 had a higher incidence of autoimmune rather than infiltrative cytopenias.[33] Taken together, the findings raise the possibility that CLL patient with +12 may be at increased risk to develop autoimmune thrombocytopenia.

Most of the patients in our study (57%) required treatment during the time of observation, with a median TTT of about 3 years. A similar proportion of treated patients (50%) but with a slightly shorter TTT (14–33 months) has been described previously in a smaller series of 47 patients.[2,37] In the latter study, baseline characteristics for patients with +12 were not reported, which may account for this slight difference. In our study, a shorter TTT was observed for patients with Rai stage III–IV, palpable splenomegaly, and del(14q) on CBA. Advanced Rai stage and massive splenomegaly are established indications for therapy, so their association with a shorter TTT is not surprising.[22] It has been shown recently that del(14q), often associated with +12 as well as with unmutated *IGHV* and *NOTCH1* mutations, portends a poor prognosis.[38] The number of patients with del(14q) in our study is small. However, our findings suggest that evaluation for this abnormality, either by conventional cytogenetic analysis and/or FISH analysis, may have prognostic relevance in these patients.

Although many patients required treatment, the ORR to first-line therapy was 94% and the median FFS has not been reached yet. Factors associated with a shorter FFS were response other than CR and FISH negativity for del(13q). The quality of the response to therapy is an independent prognostic factor in CLL, demonstrated by recent studies analyzing the importance of minimal residual disease eradication.[39,40] Further studies are needed to shed light on the biological mechanisms favoring a longer FFS when del13q and +12 are concomitant.

Similarly, median OS has not been reached in our analysis and only 18 patients died during the time of observation. Other studies have reported similar results, with a median OS of 133 months despite a significantly shorted median TTT.[2,7,41] Surprisingly, the mortality of patients with +12 was only partly related to complications of CLL; the leading causes of death were RT and SMN. In our series, RT was observed in 2% of patients with +12, in line with other large retrospective analyses.[42] However, this was significantly higher than in patients with negative FISH (0.4%), as partially reported in other series.[13,43] Though some

hypothesis have been done, the mechanism linking +12 to RT still remains unclear.[14] Similarly, in our study the incidence of SMN among patients with +12 was 9%, significantly higher than in patient with negative FISH (1%). A correlation between CLL and SMN has been reported previously[44], though a specific association with +12 is a novel finding. The mechanism linking +12 to the onset of SMN remains unclear. However, given the recent evidence of a worse outcome for SMN in CLL[45], increased surveillance of patients in this specific group should be considered.

It is interesting that the MVA for TTT, FFS and OS revealed different factors, and that not a single variable appeared to be associated uniformly with survival. CLL +12 has a higher expression of CD20, probably determining a better response to R-based regimens.[46] Factors determining CD20 expression may influence response to treatment, and be totally independent from factors determining disease progression to first therapy (presence of del14q). At the same time, unless the burden of disease is high (elevated ALC), a deeper response to therapy may be achieved, and subsequently OS may be determined by other factors (such as second cancers).

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Clinical Practice Point

- Patients with trisomy 12 (+12) CLL have a higher incidence of thrombocytopenia, Richter Transformation (RT) and second cancers, in addition to the expected increased rate of CD38 positivity and atypical immunophenotype
- Time to first treatment is shorter in patients with advanced Rai stage, splenomegaly, and deletion 14q by cytogenetic.
- Failure-free survival is shorter in patients who don't achieve complete remission or in absence of deletion 13q by fluorescence in situ hybridization.
- OS is shorter in patients with lymphocytosis and who developed SMN
- Mortality rate is low (7%) and mostly related to RT and SMN
- Increased surveillance for second cancers is needed in this group

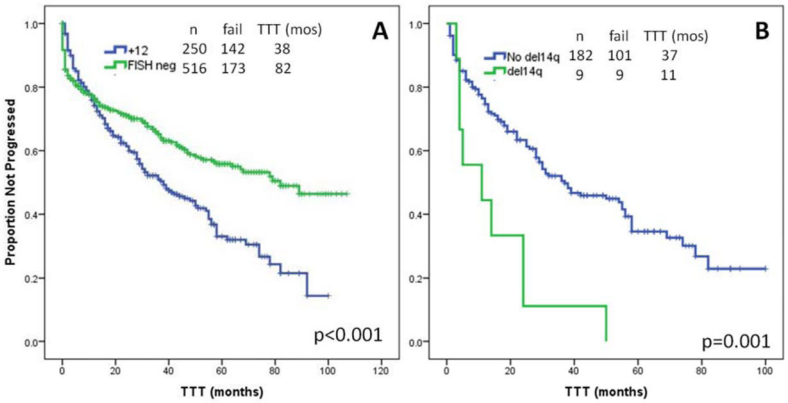

Figure 1.

Time-to-first-treatment (TTT). A. The median TTT was significantly shorter for patients with +12 than for patients with negative FISH (38 vs 82 months, p<0.001). B. On MVA, CBA positivity for del(14q) was associated with a significantly shorter TTT (11 vs 37 months, p=0.001).

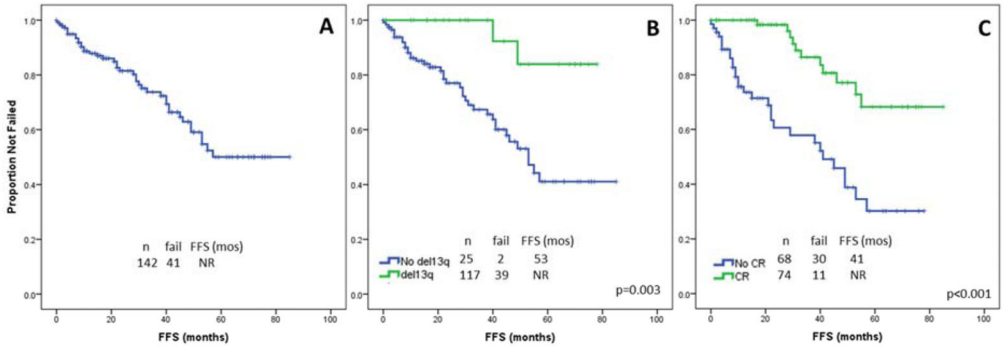

Figure 2.

Failure-free survival (FFS). A. With a median follow-up of 22 months (range, 1–85), the median FFS following first-line treatment has not been reached. B. On MVA, FISH negativity for del(13q) was associated with a significantly shorter FFS (53 months vs not reached, p=0.003). C. On MVA, response other than CR was associated with a significantly shorter FFS (41 months vs not reached, p<0.001).

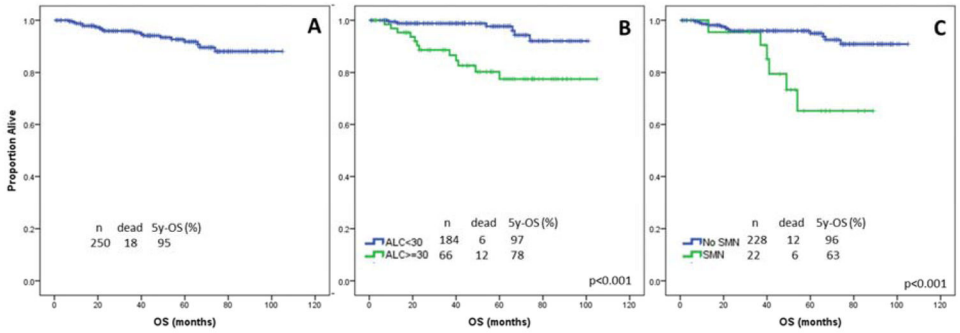

Figure 3.

Overall survival (OS). A. With a median follow-up of 51 months (range, 1–105), the median OS has not been reached. B. On MVA, ALC ≥30 $X10^9$/L was associated with a significantly shorter 5-year OS (78% vs 98%, p<0.001). C. On MVA, onset of SMN was associated with a significantly shorter 5-year OS (63% vs 96%, p<0.001).

Table I

Patients with +12 by FISH and/or CBA*

Patients (N=250)	Number (%)
FISH+/CBA−	100 (40)
FISH+/CBA+	91 (36)
FISH+ only	59 (24)
FISH +12: alone	206 (82)
with del(13q)	44 (18)
FISH +12: 7–25%	56 (22)
26–50%	86 (34)
51–75%	94 (38)
76–100%	14 (6)
CBA: diploid	93/191 (47)
+12 alone	40/191 (21)
with +19	14/191 (7)
with del(14q)	9/191 (5)
with +18	6/191 (3)
others§	29/191 (17)

*Cases were classified using the hierarchical risk model of FISH anomalies; thus cases with concomitant del(11q) or del(17p) were not included in the analysis.

§Additional chromosomal abnormalities associated with +12 identified by CBA and with a frequency <3% included +8, del13q and t(14;19)(q32;q13).

Abbreviations: FISH, fluorescence in situ hybridization; CBA, chromosome banding analysis.

Table II

Baseline characteristics and comparison to patients with negative FISH

Characteristics	+12 (N=250)	neg (N=516)	p-uni	OR [95% CI]	p-multi
Males	147 (59%)	292 (57%)	0.59	-	-
Age ≥65 years	92 (37%)	165 (32%)	0.19	-	-
Rai stage III–IV	39 (16%)	57 (11%)	0.08	-	-
Bulky (≥5cm) lymph nodes (n)	5 (2%)	6 (1%)	0.35	-	-
Splenomegaly (n)	40 (16%)	60 (12%)	0.11	-	-
Hepatomegaly (n)	14 (6%)	32 (6%)	0.87	-	-
Hemoglobin <11 g/dL	27 (11%)	38 (7%)	0.07	-	-
Platelets <100 $X10^9$/L	37 (15%)	30 (6%)	**<0.001**	**2.4 [1.1–5.3]**	**0.03**
ALC ≥30 $X10^9$/L	66 (26%)	105 (20%)	0.06	-	-
BM-Lymphocytes ≥80%	43/199 (22%)	57/385 (15%)	**0.05**	1.3	0.35
B2M ≥4 mg/L	44/243 (18%)	60/504 (12%)	**0.02**	1.6	0.18
IGHV unmutated	103/187 (55%)	160/362 (44%)	**0.02**	1.3	0.34
CD38 ≥30%	109/198 (55%)	109/361 (30%)	**<0.001**	**2.4 [1.4–3.9]**	**0.001**
ZAP70 positive	88/195 (45%)	166/342 (48%)	0.47	-	-
VH4-34	21/151 (14%)	21/250 (8%)	0.09	-	-
VH3-23	17/151 (11%)	24/250 (10%)	0.61	-	-
Matutes score <4	90/185 (49%)	96/321 (30%)	**<0.001**	**2.4 [1.5–4.1]**	**<0.001**

Abbreviations: FISH, fluorescence in situ hybridization; neg, negative FISH; OR, odds ratio; CI, confidence interval; ALC, absolute lymphocyte count; BM, bone marrow; B2M, beta-2-microglobulin

Table III

Multivariable Model for Survival

	TTT (months)	HR	p-multi	FFS (months)	HR	p-multi	5yrs-OS (%)	HR	p-multi
Rai stage II–IV	12	3.3	0.02	-	-	-	-	-	-
Splenomegaly	13	2.3	0.007	-	-	-	-	-	-
CBA+ for del14q	11	3.5	0.004	-	-	-	-	-	-
FISH- for del13q	-	-	-	53	6.2	0.01	-	-	-
No CR	-	-	-	41	3.3	0.003	-	-	-
ALC > 30 $X10^9$/L	-	-	-	-	-	-	78	14.5	0.04
SMN	-	-	-	-	-	-	63	23.8	0.002

Abbreviations: TTT, time to first treatment; HR, hazard ratio; FFS, failure-free survival; OS, overall survival; CBA, chromosome banding analysis; FISH, fluorescence *in situ* hybridization; CR, complete remission; ALC, absolute lymphocyte count; SMN, second malignant neoplasm

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