Published in final edited form as:

Leuk Lymphoma. 2015 November ; 56(11): 3031–3037. doi:10.3109/10428194.2015.1023800.

Cytogenetic Prioritization with Inclusion of Molecular Markers Predicts Outcome in Previously Untreated Chronic Lymphocytic Leukemia Patients Treated with Fludarabine or Fludarabine Plus Cyclophosphamide: A Long-Term Follow-Up Study of the U.S. Intergroup Phase III Trial E2997

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CONFLICT OF INTEREST STATEMENT: No authors have conflicts of interest with this work.

AUTHOR CONTRIBUTIONS

DML oversaw correlative sample collection, performed molecular characterizations and wrote the paper; **ASR** conducted statistical analyses and interpretation and wrote the paper; **GL** conducted molecular analyses and wrote the paper; **GWD** performed cytogenetics; **AL**, **RC**, **CP**, **DFJ** and **JGG** performed and/or directed molecular characterizations; **IWF** led the E2997 clinical trial and contributed patients/samples; **DSN** performed E2997 statistical analyses; **EMP** and **JMB** received patient samples and performed biological characterizations; **MAH** contributed patients/samples and represents SWOG; **FRA** contributed patients/samples and represents SWOG; **RAL** contributed patients/samples and represents Alliance; **DFM** contributed patients/samples and represents Mayo Clinic; **MST** contributed patients/samples and represents ECOG; **JCB** designed the studies and is the Principal Investigator (PI) of P50 CA140158 that supported some of the molecular characterizations; **MRG** designed the studies, wrote the paper, oversaw the project, and was the PI of R01 CA88647 that funded this work. All authors approved the final manuscript.

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Abstract

Fludarabine (F) and cyclophosphamide (C) remain backbones of up-front chemotherapy regimens for chronic lymphocytic leukemia (CLL). We report long-term follow-up of a randomized F versus FC trial in untreated, symptomatic CLL (NCT00003764, clinicaltrials.gov). With median follow-up of 88 months, estimated median progression-free survival (PFS) was 19.3 versus 48.1 months for F (N=109) and FC (N=118) respectively ($p<0.0001$), while median overall survival (OS) was 88.0 versus 79.1 months respectively ($p=0.96$). In multivariable analyses, variables associated with inferior PFS and OS respectively were older age ($p=0.002$, $p<0.001$), Rai stage II ($p=0.006$, $p=0.02$) and sex ($p=0.03$, PFS only). Occurrence of del(17)(p13.1) predicted shorter PFS and OS ($p<0.0001$ for each), as did del(11q)(22.3) ($p<0.0001$ and $p=0.005$, respectively), trisomy 12 with mutated *Notch1* ($p=0.003$ and $p=0.03$, respectively) and unmutated *IGHV* ($p=0.009$ and $p=0.002$, respectively), all relative to patients without these features. These data confirm results from shorter follow-up and further justify targeted therapies for CLL.

Keywords

fludarabine; cyclophosphamide; chemotherapy; chronic lymphocytic leukemia

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is characterized by the progressive accumulation of malignant CD5+/CD19+ B-cells in the periphery, lymph nodes, spleen and bone marrow. The diversity of clinical outcomes in CLL is well established, but the factors that lead to this diversity are only partially understood. To better define CLL patient subsets that will require early treatment or will have a poor response with therapy, a number of prognostic factors have been identified that include *IGHV* gene mutational status and associated ZAP-70 expression [1–4], CD38 expression [1], interphase cytogenetic abnormalities [5], and the presence of non-silent *p53* mutations [6], among others. Although efforts are underway to incorporate such molecular and biologic characteristics into the decision to initiate therapy as part of early intervention clinical trials, outside of clinical investigation, CLL is typically treated only upon the appearance of symptoms.

For many years, initial treatment for fit CLL patients who lack the del(17)(p13.1) abnormality have typically included fludarabine (F) with or without cyclophosphamide (C) [7–10]. These agents resulted in notable improvements in response and progression-free survival (PFS) over the previous standard chlorambucil, although in the four-year follow-up analysis, they did not improve overall survival (OS) [9]. The addition of CD20-directed monoclonal antibodies such as rituximab to up-front chemotherapy regimens introduced the potential to prolong survival of patients with CLL, with manageable toxicities [11,12]. Despite these improvements, CLL remains incurable and continues to present significant clinical challenges. Recently, advancements in CLL B-cell biology prompted the

introduction of agents that inhibit the B-cell receptor pathway. Such drugs, exemplified by the Bruton's tyrosine kinase inhibitor ibrutinib [13–15] and the PI3 kinase inhibitor idelalisib [16,17], have produced impressive results in both previously treated and refractory CLL patients, with modest side effects. Other therapeutics not yet approved for marketing such as ABT-199 also have shown significant promise [18]. These data are extremely encouraging, and will likely represent the foundation of future combination strategies with biologic approaches such as monoclonal antibodies, antibody-like engineered molecules, and chimeric antigen receptor T-cells. However, at this time F and C remain central components of the standard, up-front CLL treatment regimen for non-del(17)(p13.1) and otherwise fit CLL patients [19].

We previously reported the results of a randomized Phase III clinical trial comparing F monotherapy with the FC combination in treatment-naïve CLL [ECOG 2997/CALGB 10103/SWOG E2997 (E2997)] [20]. This clinical investigation showed that the addition of C provided superior complete response (CR) rates and longer PFS over F alone without impacting the rate of infections. OS was not observed to be prolonged with the addition of C, potentially due to the relatively short follow-up. We also conducted companion studies examining an array of baseline molecular and biologic factors and their impact on the achievement of CR as well as PFS; OS data were not previously reported in the context of genetic features [21]. In those studies, cytogenetic abnormalities del(17)(p13.1) and del(11)(q22.3) were associated with reduced PFS, but not CR. These findings were not different by treatment arm. Other potential predictive markers including *IGHV* gene mutation status and expression levels of CD38 and ZAP-70 did not provide additional prognostic information with respect to CR and PFS. Here, we provide long-term follow-up information on this uniformly-treated CLL population, including investigation of new markers that have arisen since the original analysis as well as re-investigation of several key molecular markers in this context.

METHODS

Patients and samples

Patients had previously untreated CLL requiring therapy according to NCI 1996 criteria [22] and provided written, informed consent under an Institutional Review Board-approved protocol for treatment and research use of their samples. A total of 278 patients were randomized to receive F or FC in the intergroup Phase III clinical trial [20], and a subset of 235 patients participated in the correlative studies [21]. Material required to conduct additional molecular analyses for this study was available on 227 (97%) of the originally described 235 patients, and there were no significant differences in this subset relative to the entire study population [20,21]. The median age at time of enrollment was 61 (range 33–86) and 73% were male. Peripheral blood, obtained immediately prior to treatment, was shipped overnight to a central processing laboratory. Mononuclear cells were isolated using Ficoll density gradient centrifugation and cryopreserved using standard techniques.

Molecular characterizations

Methods for interphase cytogenetic analysis by Fluorescence *In Situ* Hybridization (FISH) [23], *IGHV* mutation analysis [24,25], and quantitative *ZAP-70* methylation analysis at CpG +223 [4,26] have been described. For this study, gene mutations and single nucleotide polymorphisms (SNPs) were newly examined using temperature gradient capillary electrophoresis (Transgenomic, Omaha NE). All samples with abnormal peaks were sequenced by Sanger methodology. Results were compared with human genome reference sequences (*BRAF*: GRCh37/hg19 Feb. 2009; all others: NCBI36.3/hg18 Mar. 2006) using Lasergene Software (DNASTAR, Madison WI) and BLAT searches (genome.ucsc.edu). SNPs were distinguished using dbSNP (www.ncbi.nlm.nih.gov/SNP/). Genes examined included *p53* (complete coding region), *Notch1*, *XPO1/CRM1*, *MDM2* (SNP309), *BRAF* and *BTK*.

Statistical analysis

Of the 278 patients enrolled on E2997, the 227 cases with available data were included in this study investigating the association of clinical characteristics and genetic markers with clinical outcomes. PFS was defined as the time from randomization until documented disease progression or death without progression, censoring patients alive and progression-free at the date of last reported contact. OS was defined as the time from randomization until date of death, censoring patients alive at last contact date. Associations between clinical and genetic features with PFS or OS were initially explored using Kaplan-Meier plots and differences evaluated with the log-rank test. Multivariable models were fit using Cox proportional hazards models and included a treatment effect. Other variables considered for inclusion were gender, age, Rai stage, white blood cell count, and a cytogenetic/molecular classification with five groups: del(17)(p13.1), del(11)(q22.3), trisomy 12 plus *Notch1* mutation, unmutated *IGHV*, and all others. These genetic categories were defined as such by incorporating molecular information into the already developed cytogenetic hierarchical model of Dohner *et al.* [5] through standard modeling procedures. Specifically, patients who harbored multiple genetic abnormalities including del(17)(p13.1) were classified in the del(17)(p13.1) group (n = 17). Among remaining patients, those who harbored del(11)(q22.3) with or without other genetic abnormalities were classified in the del(11)(q22.3) group (n = 35). In patients without del(17)(p13.1) or del(11)(q22.3), patients with trisomy 12 would typically be classified next. However, modeling results showed that PFS, and to a lesser extent OS, were different between patients with trisomy 12 plus a *Notch1* mutation and patients with trisomy 12 patients without a *Notch1* mutation. This led to a separate classification group of patients with trisomy 12 plus mutated *Notch1* (n = 19). In addition, modeling results showed that PFS and OS were similar among the remaining cytogenetic groups [del(6)(q23) (n = 8), trisomy 12 without *Notch1* mutation (n = 30), normal interphase cytogenetics (n = 38), and del(13)(q14.3) (n = 74)], but that PFS and OS were different according to *IGHV* status in these patients. Hence, patients without del(17)(p13.1), del(11)(q22.3), or trisomy 12 plus *Notch1* mutation were grouped as either unmutated *IGHV* (n = 57) or other (n = 66). Patients with missing FISH data (n = 5), trisomy 12 but missing *Notch1* data (n = 1), and lower-risk genetic abnormalities but missing *IGHV* data (n=27)

were not included in the cytogenetic/molecular classification. All tests were two-sided and statistical significance was set at $\alpha=0.05$.

RESULTS

Patients and treatment outcome

Here we report follow-up data from 227 patients (F=109, FC=118) with clinical and molecular data available (Table I). With median follow-up of 88 months, PFS was significantly longer with the combination versus F alone (median PFS 48.1 and 19.3 months, respectively; $P<0.0001$) (Figure 1A). However, even with this extended follow-up time, OS did not differ significantly between patients treated with FC versus F (median OS 79.1 versus 88.0 months, respectively; $P=0.96$) (Figure 1B). The finding that the addition of C to F treatment provides no OS benefit is in agreement with reports from similar studies with shorter follow-up [27,28].

Cytogenetic and molecular features

As in the first report of data from these patients [21], the Dohner hierarchical classification was initially used to prioritize patients with multiple cytogenetic aberrations by FISH. As expected, patients with $\text{del}(17)(p13.1)$ had aggressive disease ($n=17$, 8%), where all but one progressed within 4 years of treatment and few appeared to be salvaged for any length of time, as demonstrated by the steep decline in OS (Tables II, III; Figure 2). A total of 35 patients (16%) were classified as $\text{del}(11)(q22.3)$ and were previously shown to have short PFS. With extended follow-up, it is clear that these patients also have shorter OS with standard chemotherapy, although not nearly to the extent of those with $\text{del}(17)(p13.1)$. Historically, the impact of trisomy 12 on clinical outcome has been unclear. However, in this dataset, we observed a significant association between patients classified as trisomy 12 and those harboring a *Notch1* mutation ($p<0.0001$), a mutation that has been previously significantly associated with poorer outcome and is generally mutually exclusive with *p53* mutations [29,30]. Among the 29 patients (13%) with a *Notch1* mutation, 19 were classified as trisomy 12, and these patients tended to have shorter PFS and OS than the 30 patients classified as trisomy 12 without a *Notch1* mutation ($p=0.02$ and $p=0.10$, respectively). Furthermore, all but two patients with trisomy 12 and *Notch1* mutations have progressed, with clinical outcomes similar to those of patients harboring $\text{del}(11)(q22.3)$. Among patients without $\text{del}(17)(p13.1)$, $\text{del}(11)(q22.3)$, or trisomy 12 plus mutated *Notch1* and with an *IGHV* result ($n = 123$), the presence of *IGHV* unmutated disease ($n = 57$) appeared to further delineate clinical outcome (PFS: $p=0.03$, OS: $p=0.02$). Other molecular features analyzed included mutations in *p53*, *BRAF*, *XPO1/CRM1*, and *BTk*, as well as the single nucleotide polymorphism (SNP) at position 309 of *MDM2*. However, the small numbers of patients with these features precluded assessment of impact on clinical outcome.

Models combining clinical, cytogenetic, and molecular predictors of outcome

Collectively, using patterns observed in our own data and in previous studies, an enhanced classification system incorporating both cytogenetic and molecular markers formed five groups: $\text{del}(17)(p13.1)$, $\text{del}(11)(q22.3)$, trisomy 12 with *Notch1* mutation, *IGHV* unmutated disease, and lastly, those without $\text{del}(17)(p13.1)$, $\text{del}(11)(q22.3)$, trisomy 12 with *Notch1*

mutation, or *IGHV* unmutated disease. Multivariable models were fit using these categories and adjusting for treatment arm as well as other significant clinical characteristics. In the final model for PFS (Table IV), treatment with FC was significantly protective and was associated with a hazard ratio (HR) of 0.37 ($p<0.0001$). Other clinical variables associated with PFS were Rai stage (HR for stage II = 1.66; $p=0.006$), sex (HR for females = 0.66; $p=0.03$) and age (HR for 10-year increase = 1.33; $p=0.002$). The cytogenetic/molecular classification group was an independent prognostic factor, where del(17)(p13.1) was associated with a HR of 4.55 ($p<0.0001$), del(11)(q22.3) with a HR of 2.54 ($p<0.0001$), trisomy 12 plus mutated *Notch1* with an HR of 2.31 ($p=0.003$), and *IGHV* unmutated disease with a HR of 1.70 ($p=0.009$), all relative to patients without these high-risk genetics. In contrast, the model for OS (Table V) did not reveal a protective effect of FC versus F (HR=0.98; $p=0.91$). However, most other variables that impacted the modeling of PFS also impacted the modeling of OS, and included: Rai stage II (HR=1.68; $p=0.02$); increased age (HR for 10-year increase = 1.54; $p<0.001$); del(17)(p13.1) (HR=15.17; $p<0.0001$); del(11)(q22.3) (HR=2.23; $p=0.005$); trisomy 12 plus *Notch1* mutation (HR=2.10; $p=0.03$); and *IGHV* unmutated disease (HR=2.27; $p=0.002$). In both PFS and OS models, the effect of cytogenetic/molecular classification on outcome did not differ by treatment arm ($p=0.21$ and $p=0.46$, respectively). Together, these observations provide long-term validation of commonly applied prognostic markers in CLL, and highlight the need to identify new treatment strategies for patients with high-risk genetic and clinical features, particularly del(17)(p13.1).

Prognostic significance of *ZAP-70* CpG+223 methylation

ZAP-70 expression is a well-established prognostic indicator in CLL [31–33]. However, reproducible measurement of its protein or mRNA remains challenging in clinical laboratories. We [4,26] and others [34,35] have reported the prognostic value of DNA methylation in the regulatory region of *ZAP-70*. Therefore, *ZAP-70* CpG+223 methylation was quantitatively assessed in a subset of 135 patients for whom material was available; clinical as well as biological characteristics of this subset were similar to the entire study population. Using the 20% cut point recently published [4], high *ZAP-70* methylation ($n=32$) was associated with extended PFS across treatment arms ($p=0.03$), but not with OS ($p=0.30$). When incorporating *ZAP-70* methylation *in lieu* of *IGHV* mutational status in the cytogenetic/molecular classification group, the model for PFS showed that low levels of *ZAP-70* methylation in patients without the high-risk genetic abnormalities del(17)(p13.1), del(11)(q22.3), or trisomy 12 plus *Notch1* mutation were associated with a HR of 1.97 ($p=0.03$) relative to patients without these high-risk genetic abnormalities and high *ZAP-70* methylation. In the model for OS, low *ZAP-70* methylation was associated with a HR of 2.14, although this did not reach statistical significance ($p=0.08$).

DISCUSSION

Here we provide updated analyses, using data mature for both PFS and OS, to evaluate the impact of cytogenetic and molecular markers in previously untreated patients with symptomatic CLL who received F monotherapy or FC combination therapy as part of an intergroup Phase III clinical trial. Samples from this study, obtained from 227 patients prior

to treatment, were investigated for a variety of new molecular markers that have arisen in recent years as well as those with established relevance in CLL prognostication. Of these, cytogenetic evaluation by FISH provided the strongest prognostic information in a multivariable analysis. Incorporating *Notch1* mutation status into the trisomy 12 classification further improved its prognostic value [36], an example of the additional benefits provided by integrating molecular information to refine subsets and inform biological investigations [37]. Although *IGHV* unmutated disease has long been associated with inferior outcome in CLL [2], it was not significantly associated with PFS in the original analysis of this dataset. However by identifying high-risk patients with del(17)(p13.1)/del(11)(q22.3)/ trisomy 12 plus *Notch1* mutation, and comparing patients with *IGHV* unmutated versus mutated disease among the remaining patients, *IGHV* status did impact both PFS ($p=0.009$) and OS ($p=0.002$). The genetic characterizations performed here further support previous work and again indicate the necessity of improved therapies particularly for the del(17)(p13.1) patient subset, but for other subsets as well. Since the addition of monoclonal antibodies to CLL chemotherapy regimens has now been shown to improve OS [11,12] and B-cell receptor targeted agents appear poised to do the same [38], better characterization of genetic subgroups from a historical study with extended follow-up provides valuable information with which results from these new agents can be compared.

Clinical outcomes according to treatment arm in this subset of patients are in close agreement with earlier reports both from this trial [20] and from similar trials [27,28], in that the FC combination significantly extended PFS relative to F alone ($p<0.0001$), but provided no OS advantage even after extended observation ($p=0.96$). The addition of C has been also reported to increase the incidence of hematologic toxicities [20,27,28] and may increase the risk of therapy-related myeloid neoplasia (in E2997, 4.6% and 8.2% at 7 years for F and FC respectively; $P=0.09$) [39]. However, the general consensus has been that FC is superior to F for patients able to tolerate such treatments.

The failure of chemotherapy regimens (F-based and others) to prolong OS of CLL patients is recognized, although the data showing this have largely been obtained from studies with shorter follow-up. In addition to the F vs. FC studies mentioned [20,27,28], a Phase III trial in older (>65 years) previously untreated CLL patients found no difference in OS between F monotherapy and chlorambucil [40], and bendamustine failed to prolong OS relative to chlorambucil [41]. An earlier Phase III trial of previously untreated CLL patients also showed no difference in OS between F monotherapy and chlorambucil [9]. Interestingly, however, in that study a modest long-term survival advantage with F began to emerge with extended follow-up (31% of F-treated vs. 19% of chlorambucil-treated patients were alive at 8 years; covariate-adjusted $P=0.07$) [42]. In our current report, there were 193 events in 227 patients (85%) for PFS, whereas in the earlier report of E2997 in 2007, there were only 129 events in 278 patients (46%) [20]. While early estimates for PFS curves are similar, we now have much longer follow-up and better estimates for the later time points that were not previously available. In this update, the median PFS for the FC group is 48.1 months versus 19.3 months for the F group, while in 2007 the median PFS estimates for the FC and F groups were 31.6 months and 19.2 months, respectively. The longer median PFS in the current study may possibly be due to a skew in early censoring in the original analysis. In

terms of OS, there are now 125 events in 227 patients (55%), whereas there were only 55 events in 278 patients (20%) at the time of the 2007 publication. No curves were previously shown for OS, but the 2-year estimates reported were 79% for the FC group and 80% for the F group. In the current study, 2-year estimates are 83% for each group, very similar our previous report. In addition, we provide long-term survival estimates with little censoring prior to 6 years. These observations together support the value of long-term follow-up, at least in clinical studies where such analysis is feasible.

Regardless, our data confirm the lack of a significant survival advantage with chemotherapy regimens for most patients, and the strong need to incorporate non-chemotherapy agents (*i.e.* monoclonal antibodies, targeted agents) into CLL treatment to extend OS. This effort is clearly gaining substantial ground with the B-cell receptor targeted agents idelalisib [16] and ibrutinib [13] (reviewed by Cheson [38]), and the advent of chimeric antigen receptor-engineered cellular therapy offers the impressive potential to activate the immune system for long-term disease control and perhaps even eradication [43].

Our data also confirm and extend the observation that there is substantial heterogeneity in CLL patient outcomes with F-based chemotherapy regimens. These therapies are insufficient for patients with del(17)(p13.1), who progress quickly and do not achieve long-term survival; in addition, patients with del(11)(q22.3), trisomy 12 plus *Notch1* mutation, and/or unmutated *IGHV* also have reduced PFS and OS relative to patients without these high-risk genetic factors. Although such chemotherapy regimens are likely to continue to be used worldwide for some time, our results underscore the importance of moving CLL treatment away from chemotherapy toward safer, targeted agents to have the greatest impact on patient outcome.

ACKNOWLEDGMENTS

The authors would like to thank the patients and the physicians who participated in E2997 and contributed samples, and the cooperative groups (ECOG, SWOG and CALGB, now Alliance) that joined in this effort. The correlative studies described here were supported by the U.S. National Institutes of Health (NIH) (R01 CA088647 and P50 CA140158). The E2997 clinical study was coordinated by the ECOG-ACRIN Cancer Research Group (Robert L. Comis and Mitchell D. Schnall, Co-Chairs) and supported in part by NIH grants CA23318, CA66636, CA21115, CA180820, CA180794, CA16116, CA180802, CA180791, CA14958, CA11083, CA13650, CA180790, CA35431, CA31946, CA32102, CA20319, and CA17145. RC receives a fellowship from the German Research Society (DFG) and is supported by the German Cancer Aid (Max Eder stipend, DKH 110461). CP is supported by the NIH (P01 CA101956), the German Cancer Research Center, and the German Cancer Consortium. The content of this paper is solely the responsibility of the authors, and does not necessarily represent the official views of the NIH.

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Figure 1. Kaplan-Meier curves by treatment arm
A) Progression-free survival; B) Overall survival.

Figure 2. Kaplan-Meier curves by cytogenetic/molecular classification

A) Progression-free survival; B) Overall survival.

Table I

Patient Characteristics

Variable	N = 227
Median age, years (range)	61 (33 – 86)
Male, n (%)	166 (73)
Rai Stage, n (%)	
0/I	61 (27)
II	64 (28)
III/IV	102 (45)
Median hemoglobin, g/dL (range)	12.4 (4.7 – 16.3)
Median platelet count $\times 10^9/L$ (range)	157 (32 – 425)
Median leukocyte count $\times 10^9/L$ (range)	73.7 (7.4 – 542.7)
FISH abnormality prioritization, n (%)	
del(17)(p13.1)	17 (8)
del(11)(q22.3)	35 (16)
+12	50 (23)
del(6)(q23)	8 (4)
Normal	38 (17)
del(13)(q14.3)	74 (33)
Unknown	5
Notch1 mutation, n (%)	
Absent	192 (87)
Present	29 (13)
Unknown	6
IGHV status, n (%)	
Mutated	85 (44)
Unmutated	109 (56)
Unknown	33
ZAP-70 methylation, n (%)	
Methylated ($\geq 20\%$)	32 (24)
Unmethylated ($<20\%$)	103 (76)
Unknown	92
Cytogenetic/molecular prioritization, n (%)	
del(17)(p13.1)	17 (9)
del(11)(q22.3)	35 (18)
+12 with Notch1 mutation	19 (10)
Unmutated IGHV	57 (29)

Variable	N = 227
All others	66 (34)
Unknown	33

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Table II

Median Progression-Free Survival (PFS) by Genomic Features

Feature	Total No. Pts	PFS (months)	No. Pts Receiving F	PFS for Pts Receiving F	No. Pts Receiving FC	PFS for Pts Receiving FC
Cytogenetics:						
del(17)(p13.1)	17	8.9	7	8.2	10	10.8
del(11)(q22.3)	35	21.1	12	17.0	23	28.4
+12	50	24.9	28	21.2	22	56.0
del(6)(q23)	8	47.8	1	26.8	7	48.5
Normal	38	37.9	22	14.3	16	61.8
del(13)(q14.3)	74	33.4	35	22.9	39	57.6
Notch1 mutation:						
No	192	28.4	93	20.9	99	47.0
Yes	29	13.1	14	8.9	15	49.1
IGHV status:						
Mutated	85	29.7	42	21.1	43	53.8
Unmutated	109	20.9	53	14.9	56	31.4
Cytogenetic/molecular group:						
del(17)(p13.1)	17	8.9	7	8.2	10	10.8
del(11)(q22.3)	35	21.1	12	17.0	23	28.4
+12 w/Notch1 mut.	19	13.1	10	9.6	9	49.1
Unmutated IGHV	57	26.8	29	16.8	28	42.8
Other	66	38.3	34	23.5	32	67.8

Table III

Median Overall Survival (OS) by Genomic Features

Feature	Total No. Pts	OS (months)	No. Pts Receiving F	OS for Pts Receiving F	No. Pts Receiving FC	OS for Pts Receiving FC
Cytogenetics:						
del(17)(p13.1)	17	13	7	13	10	18
del(11)(q22.3)	35	70	12	NR	23	70
+12	50	88	28	88	22	85
del(6)(q23)	8	72	1	49	7	75
Normal	38	106	22	84	16	NR
del(13)(q14.3)	74	115	35	115	39	NR
Notch1 mutation:						
No	192	88	93	88	99	83
Yes	29	71	14	71	15	72
IGHV status:						
Mutated	85	91	42	97	43	91
Unmutated	109	71	53	71	56	69
Cytogenetic/molecular group:						
del(17)(p13.1)	17	13	7	13	10	18
del(11)(q22.3)	35	70	12	NR	23	70
+12 w/Notch1 mut.	19	72	10	75	9	72
Unmutated IGHV	57	77	29	71	28	77
Other	66	115	34	115	32	NR

Table IV

Multivariable Model for Progression-Free Survival

Variable	HR	95% CI	P
Treatment arm: FC vs. F	0.37	0.27–0.52	<0.0001
Age: 10 year increase	1.33	1.11–1.59	0.002
Sex: Female vs. Male	0.66	0.46–0.95	0.03
Rai stage: II/III/IV vs. 0/I	1.66	1.16–2.37	0.006
Cytogenetic/molecular group:			
del(17)(p13.1)	4.55	2.56–8.09	<0.0001
del(11)(q22.3)	2.54	1.60–4.03	<0.0001
+12 w/ <i>Notch1</i> mutation	2.31	1.33–4.02	0.003
Unmutated <i>IGHV</i>	1.7	1.14–2.53	0.009
Other (Reference)	---	---	---

Table V

Multivariable Model for Overall Survival

Variable	HR	95% CI	P
Treatment arm: FC vs. F	0.98	0.67–1.43	0.91
Age: 10 year increase	1.54	1.23–1.92	<0.001
Rai stage: II/III/IV vs. 0/I	1.68	1.07–2.65	0.02
Cytogenetic/molecular group:			
del(17)(p13.1)	15.17	7.64–30.11	<0.0001
del(11)(q22.3)	2.23	1.27–3.93	0.005
+12 w/ <i>Notch1</i> mutation	2.1	1.08–4.10	0.03
Unmutated <i>IGHV</i>	2.27	1.35–3.83	0.002
Other (Reference)	---	---	---