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FCR and Bevacizumab (FCR-B) Treatment in Patients with Relapsed Chronic Lymphocytic Leukemia (CLL)

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Abstract

Patients with relapsed chronic lymphocytic leukemia (CLL) often achieve response with chemoimmunotherapy but have short remission durations. Studies have shown that patients with CLL have increased angiogenesis in the microenvironment; levels of pro-angiogenic growth factors such as VEGF and/or angiopoietin-2 (Ang-2) are also elevated. Increased angiogenesis correlates with poor outcome in CLL. Bevacizumab (B) is a humanized monoclonal antibody targeting VEGF-A. In this study, we analysed whether a combination of bevacizumab (B) with FCR chemoimmunotherapy (FCR-B) could improve outcomes in patients with relapsed CLL. Sixty-two patients were enrolled. The median age of the patients was 60 years (range, 31–84 years) and 40% had received >1 prior therapy for CLL. Sixty-one patients were evaluable for toxicity and 57 were evaluable for response. Six cycles were planned; 36 (59%) patients completed ≥ 4–6 cycles of the regimen. The overall response rate (ORR) was 79% with 13 (23%) complete remissions (CR), 8 (14%) nodular partial remissions (nPR) and 24 (43%) partial remissions (PR). The median progression free survival (PFS) and overall survival (OS) rates were 13.5 and 45 months, respectively. Grade 3 or 4 toxicities included febrile neutropenia (n=40), infections (n=21), thrombocytopenia (n=18) and anemia (n=9). Results with FCR-B were similar to those observed with an historical cohort of relapsed patients treated with FCR.

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Authorship Contributions
S.O.B. designed the study.
P.J., M.K., H.L, W.Q., and S.O.B. analyzed results.
P.J., H.L, W.Q, O.B., and S.O.B. wrote the paper.
P.J., M.K., H.L, W.Q., and S.O.B. did the clinical correlation.
M.K., S.O.B., J.B., W.W., A.F., Z.E., contributed patients.

All authors have reviewed and contributed to the manuscript.

Introduction

Chronic Lymphocytic Leukemia (CLL) is a clonal B cell malignancy with a heterogeneous disease course. Chemoimmunotherapy is frequently used as frontline therapy in patients requiring treatment and produces high response rates and durable remissions. However, in relapsed patients subsequent response to chemotherapy-based regimens is less and response duration is significantly shorter.[1,2] Advances in the understanding of the CLL microenvironment, cell signaling, and molecular biology have helped in identifying novel therapeutic targets.[3, 4] Angiogenesis is one of the main mechanisms promoting the growth and metastasis of cancer cells.[5, 6] Studies have demonstrated evidence of active angiogenesis, elevated angiogenic factors such as VEGF (vascular endothelial growth factor), angiopoietin-2 (Ang-2)[7], overexpression of VEGF receptors and increased bone marrow microvessel density (MVD) in CLL.[8–13] Interaction of microvascular endothelial cells with CLL cells promotes the proliferation of CLL cells in the lymph nodes and the bone marrow[14], enhances their survival by NF-kappaB activation and protects CLL cells from fludarabine-induced apoptosis.[15, 16] Furthermore, an increased degree of angiogenesis as documented by high bone marrow microvessel density and elevated levels of angiogenic factors in the blood is associated with advanced clinical stage, shorter time to first treatment (TTFT) and shorter progression free survival (PFS) in patients with CLL.[17, 18] CLL cells express VEGF receptors and they can also produce VEGF under the influence of microenvironmental factors; in turn, CLL cells can promote endothelial cell proliferation.[19–21] In experiments where CLL cells were cultured with exogenous VEGF, VEGF was shown to promote the levels of antiapoptotic proteins such as MCL-1.[22]

Bevacizumab is a recombinant humanized monoclonal antibody (mAb) targeting VEGF. Clinical trials in patients with metastatic solid tumors have shown that addition of bevacizumab to chemotherapy can prolong the progression free survival (PFS).[23–30] In a randomized clinical trial, 402 patients with metastatic colorectal cancer were treated with irinotecan, 5-fluorouracil, leucovorin in combination with bevacizumab. The addition of bevacizumab to chemotherapy significantly improved the progression free survival, overall survival and duration of remission. [24] Similar results were seen when bevacizumab was combined with chemotherapy in patients with non-small cell lung cancer, cervical cancer, ovarian cancer and metastatic breast cancer. [25, 28, 31, 32]

Robak et al [33] reported on the use of fludarabine, cyclophosphamide and rituximab (FCR) as a treatment for relapsed CLL in a randomized study which compared FCR to FC. Eighty two percent of these patients had been previously treated with an alkylating agent and only 17% had received fludarabine. The FCR regimen demonstrated an overall response rate (ORR) of 70%, complete remission (CR) rate of 24% and a median PFS of 30.6 months with a median follow up time of 25 months. We have earlier reported on the efficacy of FCR in relapsed patients with CLL.[34] Seventy percent of patients had received prior fludarabine based therapy, including four patients who had been treated with FCR previously. The ORR was 74%, the CR rate was 30%, and the median PFS was 21 months with a median follow up time of 43 months.

Given the correlation with increased angiogenesis and disease progression in CLL, we tested whether the addition of bevacizumab to FCR could improve PFS in patients with relapsed CLL.

Methods

This study was approved by the Institutional Review Board (IRB) of the MD Anderson Cancer Center (MDACC) and registered on (www.clinicaltrials.gov) with a trial identifier number -NCT00448019. All patients provided informed consent as per institutional guidelines and in accordance with the Declaration of Helsinki. A total of (n=62) patients were enrolled in this open-label, phase 2 trial from May 2007 through February 2010. All the patients had progressive CLL with an indication for treatment as per NCI-WG criteria. Patients had relapsed, fludarabine sensitive (duration of response >6 months) or fludarabine naïve CLL. Refractoriness to alkylating agent was defined as failure to achieve at least a partial remission (PR) with the last alkylating agent-based treatment or progression within 6 months of treatment. Patients were required to have an adequate performance status (WHO/Eastern Cooperative Oncology Group [ECOG] performance status ≤2) and adequate organ function (serum creatinine ≤2 mg/dL, total bilirubin ≤2 mg/dL, AST (SGOT) and ALT (SGPT) ≤2 times the upper limit of normal). Prognostic markers including CD38 status, presence or absence of Zap-70, immunoglobulin heavy chain variable gene *(IGHV)* mutation status, standard metaphase cytogenetic analysis and FISH- fluorescent *in-situ* hybridization was performed on bone marrow at baseline in the majority of patients.

FCR comparison group

Patients enrolled at MDACC on a previously published clinical trial of FCR as a therapy for relapsed CLL were used as a historical comparison group (n= 205). [34] The median follow up time for the FCR cohort was 120 months (95% CI, 116 – 128 months).

Treatment Plan

FCR consisted of fludarabine (F) 25 mg/m^2 IV and cyclophosphamide (C) 250 mg/m^2 IV on days 2–4 for course 1 and days 1–3 for courses 2–6. Rituximab (R) was administered on day 1 at 375 mg/m^2 for course 1 and 500 mg/m^2 for courses 2–6. Bevacizumab (B) 10 mg/kg IV was given on day 3 of course 1 and day 2 of courses 2–6. The basis for choosing bevacizumab 10mg/kg every 4 weeks was based on 10mg/kg being the most commonly used dose as well as 4 weeks being the established schedule of FCR administration. Courses were repeated every 4 weeks or longer depending on the recovery of neutrophil and platelet counts. Dose reductions for FC, but not R or bevacizumab, occurred if the patients experienced prolonged grade 3 or 4 hematologic toxicity or infections. Prophylaxis against tumor lysis syndrome, herpes virus reactivation, *Pneumocystis jiroveci* pneumonia, and the use of myeloid growth factors were at the discretion of the treating physician.

Response assessment

Patients were evaluated for response according to 1996 NCI-WG response criteria at least 2 months after their last course. [35] Computerized tomography (CT) scans were not routinely performed for response assessment. Adverse events (AEs) and serious adverse events

(SAEs) were documented according to the Common Toxicity Criteria (CTC) Version 2.0 (National Cancer Institute).

Statistical considerations

The primary objective was to determine PFS and duration of response to FCR-B in previously treated patients with CLL. Secondary objectives were to assess the response rates, CR rate, ORR and safety of FCR-B. PFS was defined as the time from the start of treatment to progression which included treatment failure, relapse or death. OS was defined as the time from the start of treatment to the last follow-up date or death. The distribution of continuous variables was summarized by mean, standard deviation and range. The distribution of each categorical variable was summarized in terms of its frequencies and percentages. Continuous variables were compared between treatment groups by Wilcoxon rank sum test, and categorical variables were compared between treatment groups by Fisher exact test. The Kaplan-Meier curve was used to estimate OS and PFS. Log rank tests were used to compare time-to-event variables between groups. The Cox proportional hazard model was used to evaluate the ability of the covariates to predict PFS or OS. All computations were carried out in SAS version 9.1 and S-Plus version 8. Kaplan-Meier curves were prepared by graph pad prism version 6.0.

Results

Patient Characteristics

62 patients with relapsed CLL were enrolled in this trial. There were 49 (79%) males and 13 (21%) female patients. The median age was 60 years (31–84 years). The median number of prior regimens was 2 (range 1–5). Five patients (8%) were previously treated with FCR. Of the total of 62 patients, 11(18%) were fludarabine and alkylating agent refractory, one (2%) was fludarabine refractory and 3 (5%) were alkylating agent refractory. Rai stage 3–4 disease was present in 58% of patients. Unmutated IGHV was observed in 83% of patients. Deletion 17p or deletion11q FISH abnormality was detected in 16% and 44% patients respectively. Among the 62 patients, 28 (45%) patients have died with a median follow-up time of 40 months (95% CI 33 – 45 months). A total of 5 patients were not evaluable (NE) in the FCR-B cohort, one patient went to hospice after one cycle due to metastatic squamous cell cancer, one patient went home after one cycle and did not return by choice, one patient declined further treatment due to grade 3–4 nausea and vomiting during first course and two patients died, one due to cardiac arrest another of an unknown cause. Two patients who died early in the course of FCR-B had preexisting cardiac co-morbidities. In one patient who died of cardiac arrest, there was a history of coronary artery disease, atrial fibrillation and anti-anginal medications. Another patient whose cause of death was unknown was a heavy smoker and had a past history of coronary artery bypass surgery. Table -1 shows the characteristics of the patients enrolled in the FCR-B trial as compared to patients treated with FCR. A significantly higher proportions of patients in FCR-B exhibited an unmutated IGHV gene, Zap-70 positivity and deletion17p or deletion11q by FISH analysis as compared to those patients in FCR cohort ($P<0.05$). The median number of cycles completed by patients in the FCR-B and the FCR trials was 4 and 5 respectively. Among the patients in the FCR-B group, 27 (44%) completed all 6 cycles. In the FCR group 91 (44%) patients

completed all 6 cycles. In relapsed patients previously treated with FCR, there were 8 early deaths– 4 after cycle one, 2 after cycle two and 2 after cycle three. The median follow-up for patients in the FCR-B and FCR trials is 40 months and 120 months respectively.

Responses

Fifty-seven of 62 patients were evaluable for response assessment to FCR-B. Among the patients evaluable for response - 8 (14%) were fludarabine and alkylating agent refractory (AFR) and 2 (4%) were alkylating agent refractory. Responses were evaluated at least 2 months after completion of therapy to allow for recovery of peripheral blood counts. Table 2 shows the overall response rate by pretreatment characteristics. The response rates with FCR-B included CR in 23% (13/57), nodular partial response (nPR) in 13% (8/14) and PR in 43% (24/43) of patients for an ORR of 79% (45/57). Five (8%) inevaluable patients came off the study early in the course of the treatment. Two patients died after cycle 2 - one patient died of cardiac arrest, and one patient died of an unknown cause. Three patients came off study after the first course, one went to hospice due to metastatic squamous cell cancer, one patient withdrew consent after a significant infusion reaction to rituximab and grade 3–4 nausea and vomiting during the first cycle and one patient went home and came off study by choice.

Patients with deletion17p by FISH also had a lower ORR as did the patients without del17p - 7% (4/57) vs 72% (41/57) respectively (P=0.03).

Response rates with FCR-B were similar to those observed with FCR in relapsed patients with CLL, CR rate (23 vs 30 %) and ORR (79 vs 78 %) respectively.

Toxicities

Sixty-two patients were evaluable for toxicity. A summary of toxicities is presented in Table 3. The most common side effects were myelosuppression, infection, nausea and vomiting. Grade 3 or 4 toxicities included febrile neutropenia (n=40), infections (n=21), thrombocytopenia (n=14), anemia (n=9) and nausea and vomiting (n=5). Grade 3–4 toxicities observed in FCR-B were similar to those seen in the historical cohort of patients treated with FCR. In the historical cohort treated with FCR, grade 3–4 pneumonia and sepsis were the most common serious infections n=46 (16%) and in patients with FCR-B, grade 3–4 pneumonia and sepsis were observed in 11 patients (18%). Potential bevacizumab associated toxicities[36] included hypertension in one patient, gastrointestinal hemorrhage in two (without perforation) and rash in two patients. All of these toxicities were grade 1–2.

Progression free survival (PFS)

PFS was analysed among patients receiving FCR-B and compared to that of the historical cohort of patients who were treated with FCR. The Kaplan-Meier estimates of PFS according to treatments (FCR-B vs. FCR) are shown in Figure 1. The median PFS was 13.5 months with FCR-B versus 21 months with the FCR regimen (P=0.05). Of note is that the FCR-B trial included a higher proportion of patients with poor prognostic characteristics, including complex karyotype, del17p or del11q, and unmutated IGHV as compared to those in the FCR trial (Table -1). These characteristics are known to shorten the PFS seen with

FCR. The median PFS by response (CR, nPR and PR) in FCR-B was 42, 22 and 12 months respectively while in the historical cohort the corresponding values were 49,29 and 15 months respectively. Multivariate analysis showing factors correlating with PFS are shown in Table -4. Factors in the multivariate model predicting for shorter PFS with FCR or FCR-B included ≤ 3 cycles of therapy, alkylating agent and fludarabine refractoriness, and deletion 11q or complex karyotype. After accounting for differences in pretreatment characteristics, treatment was not significant in the multivariate analysis.

The impact of cytogenetics by FISH on PFS in patients who received FCR-B was analysed (Figure -2). The median PFS (months) in patients with del17p, normal FISH, del11q and del13q categories were 3, 10, 17 and 22 months respectively (Log rank p=0.037). There were 3 patients with trisomy 12 and only one has relapsed.

Overall survival (OS)

Overall survival (OS) was analysed among the patients receiving FCR-B and compared to that of patients treated with FCR. The Kaplan-Meier estimates of OS according to treatment (FCR-B vs. FCR) are shown in Figure 3. The median OS was 45 months in FCR-B versus 49 months in FCR regimen (P=0.48). The median OS by response (CR, nPR and PR) in patients who received FCR-B was not reached, not reached and 34.3 months respectively while in the historical cohort the corresponding values were 129, 100 and 42 months respectively. Multivariate analysis showing factors predicting for overall survival is summarized in Table -5. Pre-treatment characteristics significantly associated with shorter OS included >2 prior therapies, ≤ 3 cycles of treatment, alkylating agent and fludarabine refractoriness, del17p by FISH and complex karyotype. Of note, treatment with FCR-B or FCR was not predictive of OS in the final Cox regression model (Table 5).

The impact of FISH categories on OS in patients who received FCR-B was analysed (Figure-4). Patients with del17p had inferior survival. The median OS (months) in patients with del17p, normal FISH, del11q, trisomy 12 and del13q categories were 7.3, 33, not reached, 34.3 and not reached respectively (Log rank P=0.0015).

Discussion

Management of relapsed patients with CLL remains a challenge. Relapse and disease progression in patients with CLL who are treated with chemotherapy can occur due to clonal evolution of CLL cells[29], persistence of drug resistant clones, presence of minimal residual disease (MRD) and pro-survival signals to CLL cells from the microenvironment. Antiangiogenic strategies have involved treatment with a monoclonal antibody against VEGF such as bevacizumab or aflibercept, or treatment with tyrosine kinase inhibitors such as sorafenib, sunitinib or pazopanib in the treatment of various solid tumors. Various randomized clinical trials have demonstrated that the combination of bevacizumab with chemotherapy in metastatic solid tumors can improve progression-free and overall survival. [30] The relevance of angiogenesis in CLL progression generated the rationale of adding bevacizumab to chemotherapy. The primary objective of this trial was to assess the outcome after the addition of bevacizumab to FCR in relapsed patients with CLL. The results using FCR-B were compared with the results of our previous trial of FCR in relapsed

CLL. The addition of the bevacizumab to FCR did not improve the PFS or OS. The FCR-B regimen was similar to FCR in terms of response rates (ORR and CR - 79% vs 78% and 23% vs 30%) respectively. The FCR-B trial had a higher proportion of patients with adverse prognostic features such as unmutated *IGVH*, complex karyotype, del11q and del17p. The majority of the patients in the historical cohort did not have FISH data available and only had karyotype analysis performed by conventional cytogenetics. In the multivariate analysis the type of therapy was not significant in predicting PFS or OS. Similar to FCR, FCR-B regimen did not improve PFS or OS in patients with del17p. Bevacizumab has been associated with cardiac and vascular toxicities. These toxicities were observed in 10–35% patients who received bevacizumab in combination with chemotherapy to treat various solid tumors. In this study potential bevacizumab associated toxicities were mild - hypertension in one patient, gastrointestinal hemorrhage in two (without perforation) and rash in two patients. This reduced toxicity in the current trial may be explained by the less frequent dosing of bevacizumab – once every 2 weeks in other studies vs once every 4 weeks in the current trial.

Bevacizumab was also evaluated in other hematologic malignancies. In a randomized phase 2 trial in elderly patients with acute myelogenous leukemia (AML), the addition of bevacizumab to chemotherapy (3+7) did not result in improved clinical benefit.[37] In non-Hodgkin's lymphoma (NHL), the addition of bevacizumab to CHOP chemotherapy alone or to rituximab-CHOP did not result in any benefit.[38, 39] In the only prior trial conducted in CLL, 12 patients with relapsed disease were treated with single agent bevacizumab and none responded. [40]

A possible explanation for the lack of efficacy of bevacizumab in hematological malignancies as compared to solid tumors could be due to less dependency of hematological malignancies on VEGF signaling and rapid adaptation of malignant cells to VEGF inhibition. Other reasons for the lack of improved PFS with FCR and bevacizumab included the fact that the status of angiogenesis (serum VEGF, Ang-2 levels, amount of bone marrow microvascular density etc.) was not evaluated in patients before therapy. It is possible that the addition of bevacizumab might be selectively useful for patients with increased levels of angiogenesis in the microenvironment.

In summary, adding bevacizumab to FCR in patients with relapsed CLL did not improve outcome and results were similar to those seen previously with FCR.

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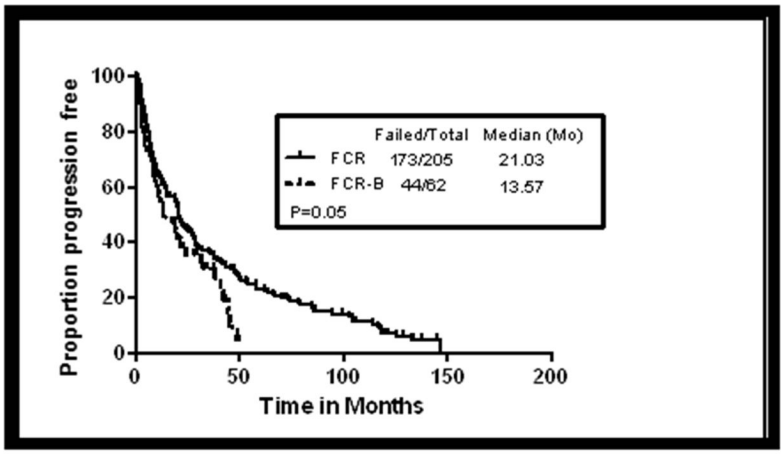

Figure 1. Progression free survival (PFS) by therapy in patients receiving FCR-B and FCR
Shows comparison of FCR-B with the historical cohort of patients who were treated with salvage FCR (P=0.05).

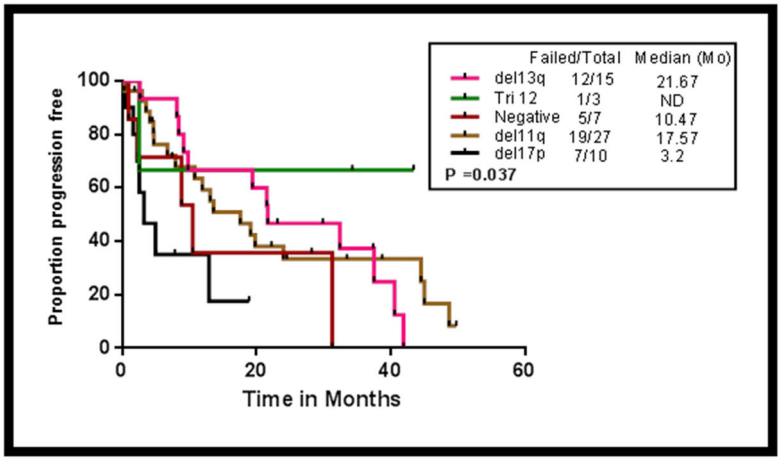

Figure 2.

Progression free survival (PFS) by different FISH (Fluorescent *in-situ* hybridization) cytogenetic categories (del17p, del11q, negative FISH, trisomy 12 and del13q) in patients receiving FCR-B (P=0.037).

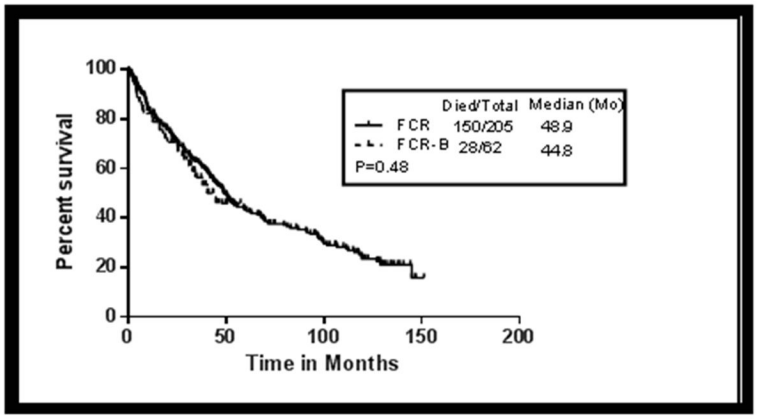

Figure 3. Overall survival (OS) by therapy in patients receiving FCR-B and FCR

Shows comparison of FCR-B with the historical cohort of patients who were treated with salvage FCR (P=0.48).

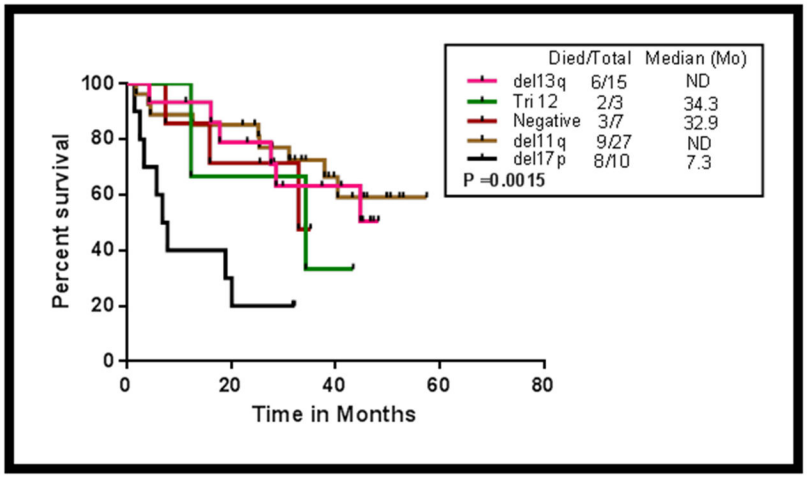

Figure 4.

Overall survival (OS) by different FISH (Fluorescent in-situ hybridization) cytogenetic categories (del17p, del11q, negative FISH, trisomy 12 and del13q) in patients receiving FCR-B (P=0.0015).

Table 1

Patient characteristics by treatment – FCR vs FCR-B

Variable		FCR (n=205) Median (range)	FCR-B (n=62) Median (range)	P-value
WBC (K/uL)		46 (1,583)	42(4, 326)	0.77
Hemoglobin (gm/dL)		12 (7,17)	12 (9,15)	0.92
Platelet (K/uL)		127 (6,391)	110 (50,338)	0.22
Variable	**Status**	**N (%)**	**N (%)**	
Age (years)	<65	137(67)	35(56)	0.17
	≥65	68(33)	27(43)	
Sex	F	57(28)	13(21)	0.33
	M	148(72)	49(79)	
Prior number of Therapies	1	82(40)	25(40)	0.93
	2	53(26)	14(23)	
	3	37(18)	13(21)	
	>3	33(16)	10(16)	
Rai Stage	0,1,2	113(55)	26(42)	0.08
	3,4	92(45)	36(58)	
***IGHV* Mutation**	Mutated	19(30)	6(17)	<0.001
	Unmutated	45(70)	29(83)	
Zap-70	Negative	24(46)	11(24)	0.02
	Positive	28(54)	34(76)	
β2 microglobulin (mg/L)	Low (<4)	78(39)	28(49)	0.17
	High (≥4)	123(61)	29(51)	
***LDH 618 IU/L (ULN)**	<618	115(58)	31(50)	0.31
	≥618	84(42)	31(50)	
ALC (K/uL)	<100	156(76)	45(73)	0.62
	≥100	49(24)	17(27)	
Nodes	<10cm	184(91)	61(98)	0.08
	≥10cm	17(8)	1(2)	
Conventional Karyotype	Del13q	6 (3)	1 (2)	0.07
	Diploid	108 (54)	27 (66)	
	Del11q	17 (8)	6 (15)	
	Trisomy 12	18 (9)	1 (2)	
	Del17p	6 (3)	2 (5)	
	Complex	23 (11)	6 (15)	
	Others	23 (11)	0 (0)	
FISH Categories**	Del13q	5 (16)	15 (24)	0.02
	Trisomy 12	8 (26)	3 (5)	
	Negative	7 (23)	7 (11)	

Variable		FCR (n=205) Median (range)	FCR-B (n=62) Median (range)	P-value
	Del11q	8 (26)	27 (44)	
	Del17p	3 (10)	10 (16)	
Alkylating agent, fludarabine refractory	No	176(86)	51(82)	0.48
	Yes	29 (14)	11 (18)	
Fludarabine refractory	No	176(86)	61(99)	0.09
	Yes	0(0)	1 (2)	
Alkylating agent refractory	No	151(74)	59(96)	0.04
	Yes	25(13)	3(5)	

*ULN-Upper limit of normal level of LDH (Lactate dehydrogenase),

**Only 31 patients treated with FCR had FISH analysis before treatment

Table 2

Overall response rate (ORR) after FCR-B by patient characteristics

Variable	Subcategory	Number	OR (%)	P value
Age	<65	32	28(88)	0.12
	≥65	24	17(71)	
Rai Stage	0–2	26	22(85)	0.33
	3–4	31	23(75)	
Bulky Disease	No	56	44(79)	1.0
	Yes	* NA	1(2)	
β2-microglobulin, mg/L	<4.0	27	23(86)	0.16
	≥4.0	26	18(70)	
Prior treatment	≤2	36	31(87)	0.08
	>2	21	14(67)	
ZAP 70	Negative	10	9(90)	0.27
	Positive	30	22(74)	
***IGHV* Mutation Status**	Unmutated	25	18(72)	0.56
	Mutated	6	5(84)	
Alkylating agent, Fludarabine refractory	No	49	40(82)	0.21
	Yes	8	5(63)	
Alkylating agent refractory	No	47	39(80)	0.23
	Yes	2	1(2)	
FISH Category	Del13q	15	13(87)	0.05
	Trisomy 12	3	3(100)	
	Negative	6	3(50)	
	Del11q	25	22(88)	
	Del17p	8	4(50)	

* NA – Not assessable

Table 3

Toxicities among the patients who received FCR-B

Toxicity	All Grades N (%)	Grades 3–4 N (%)
Neutropenia	41 (67)	40 (65)
Thrombocytopenia	18 (29)	14 (23)
Infection	30 (49)	21 (35)
Nausea and vomiting	22 (36)	5 (8)
Fatigue	16 (26)	0
Anemia	9 (15)	9 (15)
GI bleeding	2 (4)	0
Rash	2 (4)	0
Blurred vision	2 (4)	0
Syncope	1 (2)	0
Hypertension	1 (2)	0

Table 4

Cox proportional hazard model for progression free survival (PFS) for patients who received FCR-B and FCR

Analysis	HR (95% CI)	P-value
Cycle (>3 vs.≤ 3)	0.43 (0.32–0.58)	<0.0001
* AFR (Yes vs. No)	2.35 (1.56–3.53)	<0.0001
** Complex vs. Diploid (CG)	2.92 (1.83–4.65)	<0.0001
Del 11q vs. Diploid	1.70 (1.04–2.78)	0.03
Treatment (FCR-B vs. FCR)	1.28 (0.88–1.86)	0.19
β2 microglobulin (mg/dL) (≥4 vs. <4)	1.10 (0.80–1.5)	0.57
Prior treatment (>2 vs.≤ 2)	1.16 (0.84–1.59)	0.37
Rai stage (3,4, vs. 0,1,2)	1.27 (0.90–1.79)	0.18
# Del 17p vs. Diploid (FISH)	1.64 (0.50–5.34)	0.41
# Trisomy12 vs Diploid (FISH)	0.74 (0.41–1.31)	0.30

* AFR refers to fludarabine and alkylating agent refractory disease,

** CG: conventional cytogenetics,

FISH: fluorescent in situ hybridization

Table 5

Cox proportional hazard model for overall survival (OS) for patients who received FCR-B and FCR

Analysis	HR (95% CI)	P-value
Cycle (>3 vs. ≤3)	0.50 (0.36, 0.70)	<0.0001
# Del 17p vs. Diploid (FISH)	4.43 (1.74, 11.32)	<0.01
* Complex vs. Diploid (CG)	2.39 (1.45, 3.94)	<0.01
Prior treat (>2 vs. <=2)	1.61 (1.13, 2.28)	0.01
** AFR (Yes vs. No)	1.64 (1.06, 2.55)	0.03
Age 65 Yrs (≥65 vs. <65)	1.39 (0.99, 1.95)	0.06
Sex (Male vs. Female)	1.44 (0.99, 2.11)	0.06
Treatment (FCR-B vs. FCR)	1.19 (0.74–1.92)	0.46
β2 microglobulin (mg/dL) (≥4 vs. <4)	1.24 (0.83–1.84)	0.29
Rai stage (3,4, vs. 0,1,2)	1.27 (0.90–1.79)	0.18
Bulky nodes (≥10cm vs. <10cm)	1.24 (0.70–2.19)	0.47
# Trisomy12 vs Diploid (FISH)	0.64 (0.32–1.25)	0.19
# Del 11q vs. Diploid (FISH)	0.96 (0.55–1.68)	0.89
LDH (≥618 vs <618 IU/L)	1.27 (0.90–1.80)	0.18

#FISH: fluorescent in situ hybridization,

*CG: conventional cytogenetics,

**AFR refers to fludarabine and alkylating agent refractory disease,