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ORIGINAL REPORT

Rituximab, Fludarabine, Cyclophosphamide, and Mitoxantrone: A New, Highly Active Chemoimmunotherapy Regimen for Chronic Lymphocytic Leukemia

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ABSTRACT

Purpose

The addition of monoclonal antibodies to chemotherapy has significantly improved treatment of chronic lymphocytic leukemia (CLL). Based on excellent results with the chemotherapy-only regimen fludarabine, cyclophosphamide, and mitoxantrone (FCM), we built a new chemoimmunotherapy combination—rituximab plus FCM (R-FCM). We report a phase II clinical trial consisting of an initial treatment with R-FCM followed by rituximab maintenance.

Patients and Methods

Seventy-two untreated CLL patients age 70 years or younger received rituximab 500 mg/m^2 on day 1 (375 mg/m^2 the first cycle), fludarabine 25 mg/m^2 IV on days 1 to 3, cyclophosphamide 200 mg/m^2 on days 1 to 3, and mitoxantrone 6 mg/m^2 IV on day 1, given at 4-week intervals with up to six cycles supported with colony-stimulating factor. Patients achieving response received maintenance with rituximab 375 mg/m^2 every 3 months for 2 years.

Results

The overall response, minimal residual disease (MRD) –negative complete response (CR), MRD-positive CR, and partial response rates were 93%, 46%, 36%, and 11%, respectively. Severe neutropenia developed in 13% of patients. Major and minor infections were reported in 8% and 5% of cycles, respectively. Advanced clinical stage, del(17p), or increased serum β2-microglobulin levels correlated with a lower CR rate.

Conclusion

R-FCM is highly effective in previously untreated CLL, with an 82% CR rate and a high proportion of MRD-negative CRs (46%). Treatment toxicity is acceptable. Parameters correlating with a lower response rate were advanced clinical stage, high serum β2-microglobulin levels, and del(17p). Based on these results, R-FCM warrants further investigation in randomized clinical trials.

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INTRODUCTION

Chronic lymphocytic leukemia (CLL) is characterized by the relentless accumulation of monoclonal CD5+ B lymphocytes in blood, bone marrow, and lymphoid tissues. The disease predominates in older individuals and its incidence increases with age. The median survival of patients with CLL is around 10 years but the individual prognosis is highly variable. Despite some advances in its therapy, CLL remains incurable with conventional treatments.[1-8]

In the past few years, rituximab-based chemoimmunotherapy has emerged as the more efficacious therapy for chronic B-cell malignancies, including CLL.[9-11] Despite these advances, there is no evidence yet that any of the newer and more effective treatments in terms of response rate and progression-free survival results in a longer overall survival.[3,5,8] However, newer therapies produce a substantial proportion of minimal residual disease (MRD) –negative complete responses (CRs), a situation which translates into a longer progression-free and overall survival.[7,8,12-16]

Based on several in vitro and in vivo studies by others[17] and ourselves,[18,19] we developed a combination chemotherapy including fludarabine, cyclophosphamide, and mitoxantrone (FCM) that proved to be highly effective in patients with CLL, both previously treated and untreated.[12,16,17]

As a next, logical development of this treatment program the effectiveness of the combination of rituximab with FCM (R-FCM) was investigated in a phase II clinical trial which includes two clearly separated treatment parts. First, patients were given R-FCM up to a maximum of six cycles. Second, after a careful evaluation of the response 3 months after concluding R-FCM, patients having achieved a response received rituximab maintenance (375 mg/m^2) every 3 months for 2 years. We report here the results of the first part of our study, namely the response to R-FCM.

PATIENTS AND METHODS

Patients

Between November 2005 and November 2007, 72 patients with untreated CLL were included in this study. The trial was reviewed and approved by ethical committees of all centers participating in the trial. All patients provided written informed consent before entry on this study. The diagnosis of CLL was established according to the National Cancer Institute/CLL Working Group (NCI-WG) criteria.[20]

Evaluation prior treatment included clinical history, physical examination, WBC with differential count, liver and renal function tests, Coombs' test, serum lactate dehydrogenase, and β2-microglobulin levels. Creatinine clearance was calculated by using the Cockcroft-Gault formula.[21] Bone marrow infiltration was assessed by needle aspiration and biopsy. Fluorescent in situ hybridization (FISH) studies were performed by using the LSI p53/LSI ATM and LSI D13S319/LSI 13q34/CEP 12 Multicolor Probe Sets provided by Vysis (Downers Grove, IL) using cutoff levels as previously described.[22] ZAP-70 expression was analyzed in all patients by using the technique described by our group.[23] CD38 expression in CLL lymphocytes was considered increased when it was $\geq$ 30%. Clinical and biologic parameters were assessed during a period of 1 month before the onset of the treatment.

Inclusion criteria were age 70 years or younger, active disease according to the NCI-WG criteria,[20] and an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2. Patients with prior history of autoimmune phenomena or a positive Coombs' test, impaired renal or hepatic function, creatinine clearance inferior to 50 mililiter/min, past history for B or C hepatitis, severe concomitant diseases, or pregnancy were excluded from the study.

Therapy

Patients received rituximab (375 mg/m^2 intravenous [IV] cycle 1, and 500 mg/m^2 IV in subsequent cycles), fludarabine (25 mg/m^2 IV over 30 minutes for 3 days), cyclophosphamide (200 mg/m^2 IV over 1 hour for 3 days), and mitoxantrone (6 mg/m^2 IV over 30 minutes on day 1) as previously described.[12,16] In patients with peripheral blood lymphocyte counts higher than 25,000/μL rituximab was split in half doses given on days 0 and 1. Patients received pegylated granulocyte colony-stimulating factor the day after the R-FCM and allopurinol 300 mg daily for 5 days starting on day 1. All patients received oral trimethoprim-sulfametoxazole twice weekly up to 9 months after the end of the treatment.

Treatment cycles were repeated every 4 weeks and administered when the neutrophil count was greater than 1,500/μL and the platelet count superior to 100,000/μL. Those patients with neutropenia, thrombocytopenia, or anemia prior the onset of the R-FCM received the subsequent cycle of therapy when their neutrophil count, hemoglobin level, and platelet counts were 75% or higher than the baseline values. If 6 weeks after the previous cycle of therapy hematologic parameters did not reach the above mentioned levels, treatment was then restarted with reduced doses of FCM. However, if after this interval absolute neutrophil count was inferior to 500/μL or the platelet count inferior to the 75% of the baseline value treatment was discontinued.

Response Criteria

Patients who received three or more cycles of R-FCM were considered assessable for response. Patients receiving fewer than three cycles and withdrawing from the study due to undue toxicity were considered as experiencing treatment failure. Response was assessed 3 months after the end of the treatment using NCI-WG criteria.[20] Patients in CR with no detectable MRD were categorized as MRD-negative CR. Bone marrow evaluation was not required for patients not attaining clinical CR. Imaging studies were not used to evaluate response to therapy.

MRD assessment was centrally analyzed in the Hematopathology Unit from the Hospital Clinic of Barcelona using multiparametric flow cytometry. Both peripheral blood and bone marrow were analyzed simultaneously at the time of response evaluation. Whole peripheral blood or bone marrow samples were incubated with quadruple combinations of antibodies in a five tube combination assay with a sensitivity of 10^{-4} and analyzed following the method described by Rawstron et al.[24]

End Points and Statistical Considerations

The main end point of the first part of the trial reported here was response rate, including MRD assessment. In addition, treatment toxicity was evaluated.

The CR rate in previously untreated patients receiving FCM is 64%.[16] Using an optimal two-stage design for phase II clinical trials, the estimated sample size to observe an increase of 15% in the CR rate, with an α error of .05 and a ß error of 20%, was 67 patients. The Fisher's exact test or χ^2-tests were used to analyze the association between patient characteristics and response. All statistical tests were two sided and the significance level was .05.

RESULTS

Patients' Characteristics and Response to Therapy

Sixty-seven patients (median age, 60 years; range, 40 to 70 years) were assessable for response. Four patients were excluded from the study because they did not fulfill the inclusion criteria and an additional patient declined treatment after inclusion into the study (Fig 1). The main characteristics of the patients are presented in Table 1.

Median number of R-FCM cycles administered was 6 (range, 3 to 6); 88% of patients received the entire planned therapy. The overall response rate was 93% (95% CI, 84% to 97%). CR was obtained in 82% of patients (n = 55; 95% CI, 71% to 89%). Among them, 36% achieved MRD-positive CR (n = 24; 95% CI, 25% to 48%) and 46% (n = 31; 95% CI, 35% to 58%) MRD-negative CR. Eight patients

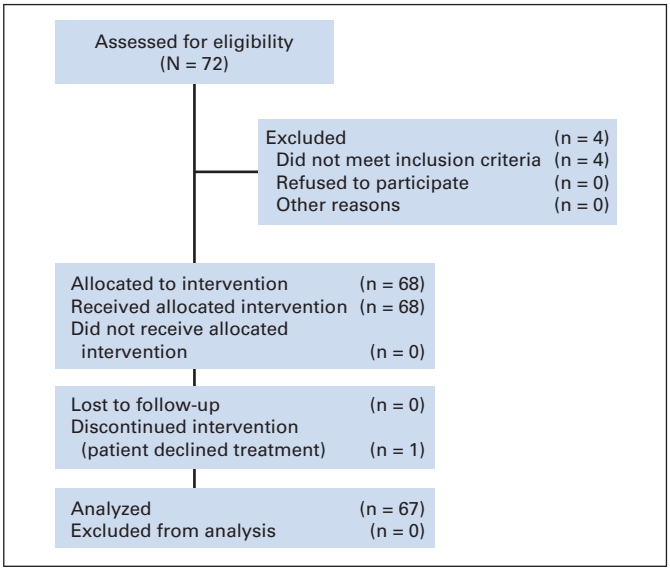

Fig 1. CONSORT diagram.

Table 1. Response Rate and Response Duration According to the Main Pretreatment Characteristics of the Patients

Variable	Distribution (%)	CR (%)	MRD-Negative CR (%)
Age, years			
Younger than 60	48	84	43
60 to 70	52	80	46
Sex			
Female	30	85	35
Male	70	81	51
Binet stage			
A	13	100	67
B	61	85*	44
C	26	65	41
Rai stage			
0	3	100	50
I-II	69	91*	50
III-IV	28	58	37
B symptoms			
No	73	80	53
Yes	27	89	28
Liver size			
Normal	45	83	46
Enlarged	55	80	47
Spleen size			
Normal	55	83	43
Enlarged	45	81	49
Lymphocyte count, μL			
$<$ 100,000	67	84	57*
$\geq$ 100,000	33	77	27
LDT (n = 57), months			
$\geq$ 6	77	86	58*
$\leq$ 6	23	69	23
Serum LDH (n = 64)			
Normal	69	84	48
Increased	31	75	40
ß2-microglobulin (n = 58), upper limit normal			
$<$ 2N	79	89*	48
$\geq$ 2N	21	72	25
BM pattern (n = 62)			
Non-diffuse	55	85	52
Diffuse	45	78	41
Genetic abnormalities (n = 58)			
del(13q)	38	82	50
+12	7	100	50
del(11q)	14	87	62
del(17p)	7	25*	0
ZAP-70 expression (n = 64)			
$<$ 20%	41	84	54
$\geq$ 20%	59	79	39

Abbreviations: CR, complete response; MRD, minimal residual disease; LDT, lymphocyte doubling time; LDH, lactate dehydrogenase; BM, bone marrow pattern.
*$P < .05$.

(11%) were considered as in PR because of persistent lymphadenopathy (two patients), incomplete bone marrow recovery (three patients) or bone marrow infiltration with lymphoid aggregates (three patients). Five patients failed (7%) to respond; in two of these patients tumor burden was not significantly reduced, two additional patients had persistent neutropenia that prevented treatment continuation, and the remaining one died due to *Aspergillus* pneumonia and cytomegalovirus reactivation.

In responding patients, a rapid tumor reduction was already observed in the first cycles of therapy. As a result, a rapid decline in blood lymphocyte count was observed with the first cycle of therapy. In addition, when analyzing the maximum clinical response not considering treatment-related cytopenias, no o little further improvement was observed beyond three cycles of therapy (Fig 2).

Pretreatment variables associated with CR are listed in Table 1. Patients with advanced clinical stage, increased serum β2-microglobulin levels, and deletion 17p were associated with a lower CR rate. Of note, only 25% of patients having a 17p deletion attained CR. No differences in response rate were observed according to age subgroups (younger than 60 *v* 60 to 70 years).

In patients obtaining CR or PR according to NCI criteria, the MRD status was investigated by flow cytometry. Among 55 patients in CR, 56% of them (n = 31) achieved MRD-negative status. Moreover, in one patient considered in PR due to the presence of lymphoid aggregates in bone marrow biopsy no MRD was detected. MRD was better detected in bone marrow than in peripheral blood, since in 77% of the patients there was a virtual absence of peripheral blood B lymphocytes.

Pretreatment variables associated with MRD-negative CR achievement were lymphocyte doubling time (< 6 *v* ≥ 6 months, 15% *v* 52%, respectively; $P = .026$) and peripheral blood lymphocyte count ($>$ 100,000 *v* $<$ 100,000/μL, 27% *v* 57%, respectively; $P = .029$). Among responding patients, cytogenetic alterations did not correlate with MRD-negative CR achievement.

Toxicity

In general, R-FCM was well tolerated. Main toxicities are detailed in Table 2. The first infusion of rituximab was fractionated in 51% of the patients as per the protocol. Infusional reaction to rituximab was observed in 37% of the patients, mainly during the first cycle of treatment. Although some degree of neutropenia was observed in 41% of cycles, severe grade 3 to 4 neutropenia was only detected in 13% of

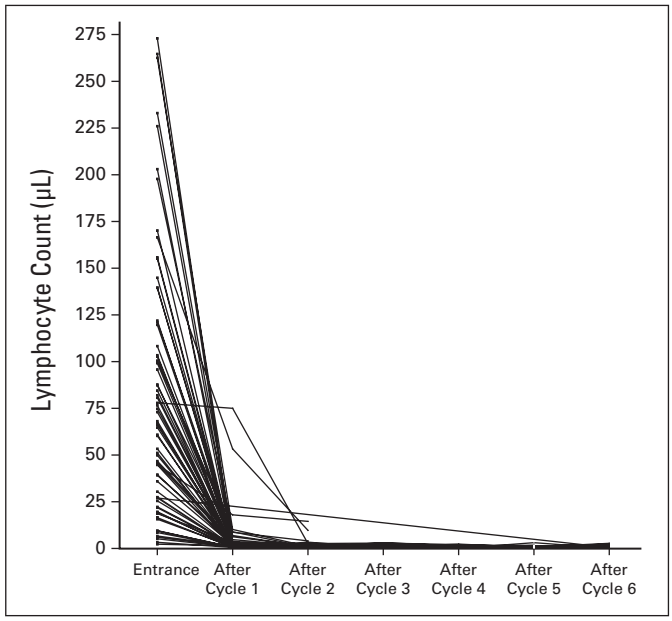

Fig 2. Blood lymphocyte counts by cycle. A rapid decline in blood lymphocyte count was observed with the first cycle of therapy.

Table 2. Hematologic and Extrahematologic Toxicity Related to the R-FCM

Toxicity	%		
	Total	Grade 1-2	Grade 3-4
Hematologic			
Neutropenia	41	28	13
Thrombocytopenia	6	4	2
Anemia	17	17	—
Nonhematologic			
Infusional reaction	10	9	1
Nausea/vomiting	14	13	1
Alopecia	2	—	2
Renal	3	3	—
Gastrointestinal	3	3	—
Hepatic	6	6	—
Infection	13	8	5
Infectious episodes by site, No.			
Fever of unknown origin		31	
%		8	
Documented infections		21	
%		5	
Pneumonia		8	
Upper respiratory tract		5	
Cutaneous infection		2	
Sepsis (*Staphylococcus* spp.)		2	
Diarrhea		1	
Herpes zoster reactivation		1	
CMV reactivation		1	
Pulmonary aspergillosis		1	

NOTE. Toxicity was based on the National Cancer Institute/Chronic Lymphocytic Leukemia Working Group and WHO classification and expressed as % of the cycles administered.

Abbreviations: R-FCM, rituximab plus fludarabine, cyclophosphamide, and mitoxantrone; CMV, cytomegalovirus.

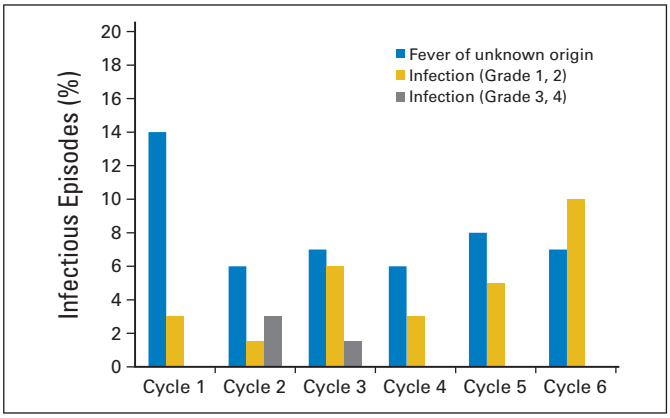

Fig 3. Percentage of infectious episodes by cycle of treatment. Blue bars represent fever of unknown origin, gold bars represent infectious episodes grades 1 to 2, and gray bars represent infections grades 3 to 4.

the cycles. The WBC nadir, analyzed during the first cycle, was observed 7 days after the onset of treatment. Grade 3/4 thrombocytopenia and anemia were observed in 2% of the cases. Dose reductions and delay on administration were necessary in 9% and 18% of the cycles, respectively, mainly because of hematologic toxicity.

Fever of unknown origin, recorded in 8% of the cycles, resulted more frequent after the first cycle. In contrast, documented infections were reported in 5% of the cycles and were more prevalent after the last cycle of R-FCM (Fig 3). One patient required admission due to a severe pneumonia complicated by cytomegalovirus and *Aspergillus flavus* infections that eventually were fatal, this resulting in a treatment-related mortality rate of 1.5%.

Neutropenic episodes were higher (57% *v* 32% of cycles, respectively; $P = .002$), and also more severe (grade 3-4 neutropenia in 22% *v* 8% of cycles, respectively; $P = .014$) in patients from 60 to 70 years of age (n = 34) than in patients younger than 60 years (n = 33). In addition, there was a trend for a higher number of infectious episodes in the subgroup of patients from 60 to 70 years of age ($P = .064$).

Treatment Results of R-FCM As Compared With FCM

Patients' characteristics and treatment results of our FCM clinical trial[16] were retrospectively compared with those of the R-FCM study reported here (Table 3). The FCM study was designed for patients younger than 65 years, whereas R-FCM included patients younger than 70 years. The remaining inclusion criteria were identical in the two treatment combinations (active CLL requiring treatment according to the NCI-WG criteria and adequate performance status). Distribution by sex, clinical stage, blood lymphocyte count, doubling time, serum β2-microglobulin levels, ZAP-70 expression in B-lymphocytes, and genetic abnormalities was not significantly different between the two groups of patients, except for a higher percentage of cases with trisomy 12 in the FCM cohort. Albeit the overall response rate was similar with the two regimens, the proportion of CRs, including those with negative MRD, was significantly higher with R-FCM than with FCM (46% *v* 26%, respectively). Also grade 3 to 4 toxicity (neutropenia, thrombocytopenia, and the number of infections) was significantly higher in patients receiving R-FCM than FCM. The R-FCM combination was also more effective and toxic than FCM when comparing patients younger than 65 years (data not shown).

DISCUSSION

This study shows that R-FCM chemoimmunotherapy is extremely active in patients with untreated CLL, with acceptable and manageable toxicity. The 93% overall response rate and 82% CR, of which 46% were MRD negative, are remarkable. Although direct comparisons of different phase II studies is inappropriate, it is of note that the CR rate found in this study is higher than those reported by the United Kingdom Leukaemia Research Fund (LRF CLL4), the German CLL Study Group, the US Intergroup with fludarabine and cyclophosphamide (FC), and pentostatin, cyclophosphamide, and rituximab (39%, 24%, 23%, and 41%, respectively).[5,8,25,26] In a large phase II study the FCR (fludarabine, cyclophosphamide, and rituximab) combination designed by the M. D. Anderson Cancer Center resulted in an overall response rate of 95% and 72% CR.[11] Although only available in abstract format, the German CLL Study Group recently reported the results of the CLL8 clinical trial comparing FCR versus FC.[27] The ORR and CR rates were higher in the FCR arm (95% and 52%, respectively) than in the FC arm (88% and 27%, respectively), with a prolongation of the progression-free survival in the FCR arm. Other trials using FCR at different doses and treatment schedules have produced CR rates ranging from 61% to 79%.[28-30] Comparing all these clinical trials, however, it is difficult because of the heterogeneity of patients included in the different studies; for example, whereas in our study no

Table 3. Comparison of the Main Characteristics of the Series and Treatment Results of Patients Included in Our Previous FCM Clinical Trial With Those of Patients Treated With R-FCM

Variable	%		*P*
	FCM	R-FCM	
No. of patients	69	67	
Age, years			
< 60	64	48	.08
≥ 60	36	52	
Sex			
Female	25	30	NS
Male	75	70	
Binet stage			
A	21	13	NS
B	67	61	
C	12	26	
Rai stage			
0	3	3	NS
I-II	74	69	
III-IV	23	28	
B symptoms			
No	67	73	NS
Yes	33	27	
Lymphocyte count, μL			
< 100,000	70	67	NS
≥ 100,000	30	33	
LDT, months			
≥ 6	60	77	.083
≤ 6	40	23	
Serum LDH (n = 65)			
Normal	77	69	NS
Increased	23	31	
ß2-microglobulin, mg/L			
< 3	49	48	NS
≥ 3	51	52	
Genetic abnormalities			
del(13q)	34	38	NS
+12	22	7	.028
del(11q)	28	14	NS
del(17p)	11	7	NS
ZAP-70 expression (n = 39)			
< 20%	44	41	NS
≥ 20%	56	59	
Grade 3-4 toxicity			
Neutropenia	4	13	< .001
Thrombocytopenia	—	2	< .01
Infection	1	5	< .001
Response			
CR MRD negative	26	46	
CR MRD positive	38	36	.034
PR	26	11	
Failure	10	7	
CR achievement predictors	Clinical stage, spleen size, serum LDH, ß2-microglobulin, BM, del(17p)	Clinical stage, ß2-microglobulin, del(17p)	

Abbreviations: FCM, fludarabine, cyclophosphamide, and mitoxantrone; R-FCM, rituximab plus FCM; NS, not significant; LDH, lactate dehydrogenase; LDT, lymphocyte doubling time; BM, bone marrow pattern; CR, complete response; MRD, minimal residual disease; PR, partial response.

patients above the age of 70 were included and only 20% of patients were older than 65 years, in other trials the proportion of patients older than 70 years is between 15% and 30%.[5,11,28]

In our study the MRD-negative CR rate was 46%. MRD status is concentrating important attention in CLL therapy since MRD-negativity is associated with a longer progression-free survival and overall survival.[7,12,16] Although the possibility of achieving MRD-negativity might be a surrogate of a less aggressive disease from the biologic standpoint, the association between MRD-negative status and a better outcome has been validated in many studies.[7,31]

Toxicity of R-FCM, mainly hematologic and infectious, was manageable and treatment-related mortality was low. The proportion of patients with severe neutropenia was lower than that reported with FCR[15] although all patients in our trial received G-CSF.

In a historical comparison of two consecutive trials conducted by our group, we found a higher CR rate with R-FCM than with FCM (64% *v* 58%), including a larger proportion of MRD-negative CRs (46% *v* 26%). Albeit this was a retrospective analysis, this comparison was performed between well-balanced subgroups of patients in terms of the main clinical and biologic variables, suggesting that the addition of rituximab highly improves the efficacy FCM. On the downside there was an increased hematologic and infectious toxicity in patients treated with R-FCM. Interestingly, in the R-FCM trial some variables indicating high tumor burden (diffuse bone marrow pattern, enlarged spleen or increased serum LDH) lost their negative predictive value for CR achievement observed in the FCM trial.

A number of other findings in our study deserve comment, particularly regarding response predictors. Of note, patients with deletion 17q responded poorly to R-FCM, this adding to the body of evidence on the ineffectiveness of fludarabine-based therapies in patients with this genetic lesion.[5,32,33] However, no differences in response rate were observed among patients with other abnormalities (ie, deletion 13q, trisomy 12, and deletion 11q). In our trial, patients' age at inclusion did not correlate with the probability of achieving CR. This is in contrast with other trials and may be explained by the absence of patients older than 70 years.[15]

Besides genetic abnormalities, clinical stage, and serum β2-microglobulin levels correlated with an inferior response. The identification of response predictors others than del(17p) is important because the proportion of patients with such a genetic abnormality is relatively low in all series.[25,34] In this regard, it is of note that serum β2-microglobulin, an easily obtainable parameter, correlated with a lower response rate both in our study and in the FCR trial recently updated by the M. D. Anderson Cancer Center Group.[11,15,34] The search for predictors for response to the newer therapies, mainly new biologic variables, is warranted.[34]

Our study has a number of limitations that deserve comment. First, results are derived from a phase II trial conducted in a relatively small number of patients. Second, since the study includes maintenance with rituximab, response duration and survival cannot be properly assessed yet. Nevertheless, the maintenance phase does not interfere with the major end point of this report which is response rate. The dramatic high CR rate obtained with R-FCM makes these results worth communicating because the ideal companion for rituximab in chemoimmunotherapy regimens for CLL is far from being established. Finally, this regimen was tested in CLL patients with a median age below that observed in standard CLL populations with no significant comorbidities. Our results showed higher hematologic toxicity

in the subgroup of patients older than 60 years. The efficacy and toxicity of this chemoimmunotherapies in elderly patients, particularly in those with comorbidities, is far from being established and warrants specific clinical trials.

In conclusion, R-FCM is a well-tolerated regimen that produces the highest CR rate reported to date, including MRD-negative CRs, in previously untreated patients with active CLL. Based on these results, randomized trials comparing R-FCM with other forms of chemoimmunotherapy are clearly warranted.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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