High clinical and molecular response rates with fludarabine, cyclophosphamide and mitoxantrone in previously untreated patients with advanced stage follicular lymphoma

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ABSTRACT

Background
Purine analogs have demonstrated significant activity in patients with follicular lymphoma. The aim of this study was to analyze the efficacy and toxicity of a fludarabine combination as first-line treatment in patients with advanced-stage disease.

Design and Methods
This is a phase II trial including 120 patients (≤65 years) treated with 6 cycles of fludarabine, cyclophosphamide and mitoxantrone (FCM). Molecular response was assessed by q-PCR in peripheral blood.

Results
Of 119 patients with an assessable response, complete response was achieved in 99 (83%) partial response in 13 (11%) and 7 (6%) did not respond. After treatment, 37 out of 46 (81%) patients achieved molecular response. After a median follow-up of 3.9 years, 32 patients have relapsed. The 5-year progression-free survival was 58% (95% confidence interval: 47-69). Variables associated with a shorter progression-free survival were a poor performance status (ECOG≥2), ≥2 extranodal sites and high β2-microglobulin. Sixteen episodes of grade 3-4 infections were observed. Two patients died during therapy (of progressive multifocal leukoencephalopathy and bronchoaspiration respectively). No late toxicity has been observed. Twelve patients died during follow-up (9 after relapse, 2 during chemotherapy, 1 in complete remission after surgery for meningioma). The overall survival at 5 years was 89%. ECOG ≥2 and high β2-microglobulin were associated with a shorter survival.

Conclusions
FCM results in high complete and molecular response rates, with prolonged response duration in younger patients with advanced-stage follicular lymphoma. The combination of FCM with rituximab as front-line treatment warrants further investigation.

Key words: follicular lymphoma, treatment, molecular response, outcome.

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Introduction

Despite the progress made in treatment, follicular lymphoma (FL) remains incurable even in patients achieving a complete response (CR). This is probably due to persistence of minimal residual disease (MRD).[1-4] The detection of *BCL2/JH* rearrangement by PCR allows us to identify patients with persistent MRD after treatment. The disappearance of *BCL2/JH* rearrangement after therapy has been associated with an improved outcome.[5-8] Purine analogs, especially fludarabine, have demonstrated significant activity in FL, particularly when combined with other agents. The pivotal study by McLaughlin *et al*. in 1994 showed that fludarabine, mitoxantrone and dexamethasone (FND) induces a high response rate in relapsed FL.[9] Similar results were reported in patients with chronic lymphocytic leukemia (CLL) and FL in progression/relapse, treated with fludarabine, cyclophosphamide and mitoxantrone (FCM).[10,11] Furthermore, in this group of heavily pretreated patients, FCM was able to induce the achievement of a molecular response in a considerable proportion of them. These clinical results were supported by *in vitro* experiments showing the synergistic effect of the combination.[12]

These promising results led the GELCAB (*Grup per l'estudi dels limfomes de Catalunya i Balears*) to conduct a phase II clinical trial to investigate the efficacy and toxicity of FCM as first-line treatment in younger patients with advanced-stage FL.

Design and Methods

Patients

Between January 2000 and December 2003, 125 previously untreated patients with FL were enrolled in this collaborative multicenter trial. The criteria for inclusion were: grade 1 or 2 FL, stage III-IV disease, age ≥18 and ≤65 years, Eastern Co-operative Oncology Group (ECOG) performance status (PS) <3, and normal renal, hepatic and cardiac function (unless secondary to FL). Patients with transformed FL were not eligible. Patients were also excluded if they were HIV positive, had a previous history of another malignancy, anemia or thrombocytopenia of immune origin, had a positive Coombs test or were receiving systemic steroids. Patients were included on the basis of the local histologic diagnosis and a subsequent central pathological review. The Follicular Lymphoma International Prognostic Index (FLIPI) could be assessed in 99 patients. The study was approved by the Institutional Review Board of each participating center and informed consent was obtained from all patients.

Table 1. Pre-treatment characteristics of 120 patients with follicular lymphoma.

	N./ N. assessable	*Percentage %*
Male gender	57/120	47
Median age, years (range)	52 (24-65)	–
Age ≥60 years	20/120	17
Histology		
Grade 1	63/118	53
Grade 2	53/118	45
Other	2/118	2
BCL2/JH breakpoint		
MBR	64/113	57
mcr	6/113	5
Not detected	43/113	38
Poor performance status (ECOG ≥2)	11/119	9
B-symptoms	27/119	23
Bulky disease (≥10 cm)#	35/120	29
Extranodal sites ≥2	35/119	29
N. nodal sites ≥5	55/89	62
BM involvement	94/120	78
Peripheral blood involvement	20/120	17
Stage IV	96/120	80
Hemoglobin <12 g/L	31/120	26
High serum LDH	21/119	18
High serum β2-microglobulin	34/115	29
FLIPI		
Low risk	21/99	21
Intermediate risk	45/99	45
High risk	33/99	33
IPI		
Low risk	58/120	48
Low/Intermediate risk	44/120	37
High/Intermediate risk	15/120	13
High risk	3/120	2

#2 mediastinal, 28 abdominal, 2 mediastinal + abdominal, 3 other; ECOG: Eastern Co-operative Oncology Group; BM: bone marrow; LDH: lactate dehydrogenase; β2m: β2microglobulin; FLIPI: Follicular Lymphoma International Prognostic Index; IPI: International Prognostic Index.

Treatment

Chemotherapy was administered on an outpatient basis every 4 weeks. Response to treatment was assessed by CT scans after the third cycle and patients not achieving CR or PR were excluded from the study. Responding patients continued treatment up to 6 cycles. Chemotherapy consisted of fludarabine 25 mg/m^2/d i.v. on days 1 to 3, cyclophosphamide 200 mg/m^2/d i.v. on days 1 to 3, and mitoxantrone 6 mg/m^2 i.v. on day 1. Subsequent cycles were administered if the neutrophil count was higher than 1.5×10^9/L and the platelet count higher than 75×10^9/L. In patients not achieving these counts at 4 weeks, treatment was delayed for 1 or 2 weeks and restarted at the initial dose. When the neutrophil count at 4 weeks was <1×10^9/L or the platelet count <50×10^9/L, the following cycle was administered at 75% of the initial dose, after the neutrophil and platelet counts had recovered. The dose was reduced to 50% of the initial dose when the neutrophil count at 4 weeks was <0.5×10^9/L or the platelet count <25×10^9/L and was administered when the neutrophil and the platelet count had recovered. When, after a 2-week delay, the counts were >1×10^9/L

and >50×10⁹/L the next cycle was given at half the initial doses. Treatment was stopped when severe (>grade 3) non-hematologic toxicity was observed. Granulocyte-colony stimulating factor (G-CSF) was administered following an episode of febrile neutropenia or after 2 cycles with grade 3-4 neutropenia regardless of dose and timing adjustments. All patients received trimethoprim-sulphametoxazol as *Pneumocystis carinii* pneumonia (PCP) prophylaxis until the CD4 lymphocyte count was higher than 0.3×10⁹/L. Toxicity was evaluated according to the WHO toxicity scale.[13] Patients in CR after completing the treatment were allowed to receive maintenance with interferon-α (IFN-α) according to the protocols of each participating center.

Assessment of response and follow-up

Response was assessed 4-8 weeks after the last cycle of chemotherapy. Complete response (CR) was defined as the disappearance of tumor masses and disease-related symptoms, as well as the normalization of initially abnormal tests and/or biopsies, lasting for at least one month. Partial response (PR) was defined as a minimum 50% reduction of measurable lesions. Patients not included in these categories were considered as non-responders (NR).[14] The follow-up surveillance policy consisted of physical examination, full blood count and biochemistry every 3 months during the first year, every 4 months during the second and third year, and every 6 months from the fourth year. Chest-abdomen-pelvis CT scans were performed every 6 months during the first year and annually for 5 years.

Assessment of BCL2/JH rearrangement and molecular response

DNA samples obtained from peripheral blood and/or bone marrow of 113 FL cases at diagnosis were assessed for the presence of *BCL2/JH* rearrangement. Conventional PCR was performed for the detection of t(14;18) using primers for *MBR* (*major breakpoint region*), *mcr* (*minor cluster region*) breakpoints at BCL-2 and the consensus *JH* primer.[15] Quantitative PCR (qPCR) analysis for rearrangement at *MBR* was also performed on PB and/or BM samples at diagnosis. qPCR was carried out in an Abi Prism 7900 thermal cycler in a total volume of 25 μL containing 500 ng of DNA, 0.125 μM/L of *JH* consensus primer and *MBR* primer and 0.15 μM/L of *MBR*-specific probe.[16] α-actin was coamplified as an internal reference. Standard curves were generated from serial dilutions of the DOHH2 cell line. The specificity of qPCR was assessed by the absence of amplification in 3 DNA samples from healthy individuals. Fifty-seven DNA samples available from the diagnostic lymph node biopsy were amplified by PCR targeting the *JH* region and distinct regions of *BCL2* gene following the BIOMED-2 protocol.[17]

Minimal residual disease was analyzed in PB at the end of therapy and one year after completing chemotherapy in responding patients. Molecular response (MR) was defined as the disappearance of *BCL2/JH* rearrangement in peripheral blood as detected by conventional and qPCR, independently of the clinical response. Minimal residual disease was not analyzed in bone marrow samples because these were not available during follow-up. Preliminary results (data not shown) supported the concordance between results analyzed in peripheral blood and bone marrow samples.[18]

Statistical analysis

The main end-point of the study was CR rate. Secondary end-points were molecular response rate, toxicity, progression-free survival (PFS), response duration (RD), and overall survival (OS). The sample size was calculated according to the optimal two-stage design for phase II clinical trials according to the fol-

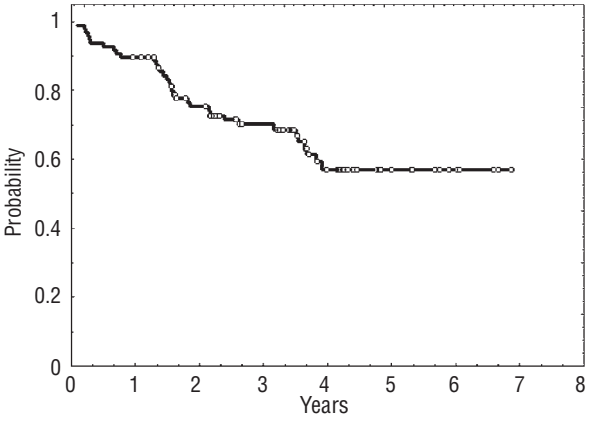

Figure 1. Progression-free survival in 98 patients with follicular lymphoma treated with the FCM regimen who did not receive additional therapy.

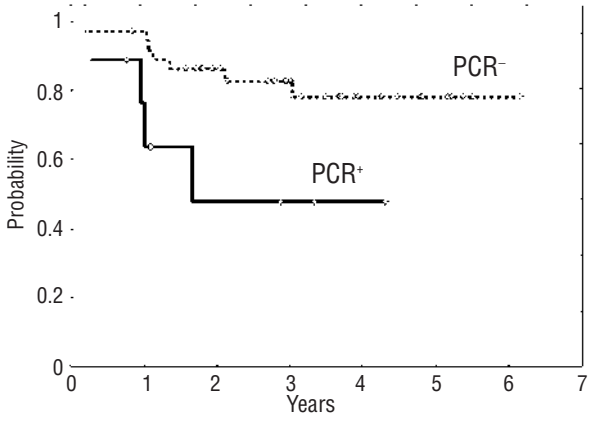

Figure 2. Response duration according to the presence or absence of *BCL2/JH* after completing chemotherapy.

lowing assumptions: P_0, 30% CR; P_1, 45% CR, with alpha and beta errors of 0.05 and 0.10 respectively.[19] PFS, RD and OS were defined according to standard criteria.[14] Actuarial survival analysis was performed according to the Kaplan-Meier method,[20] and the curves compared by the log-rank test.[21] Univariant analysis was carried out for the main characteristics at diagnosis and prognostic factors found to be significant were included in a multivariate analysis. A logistic regression model was used to identify predictive factors for CR achievement and multivariate analyses for PFS, RD and OS were performed by a stepwise proportional Cox regression model.[22]

Results

Patient characteristics and treatment

One hundred and twenty patients were included in the analysis. Five patients were excluded. Reasons for this included incorrect diagnosis (unclassifiable CD20+ B-cell lymphoma, n=1; FL with grade 3 FL, n=1), and other exclusion criteria (previous history of breast cancer, n=1; concomitant treatment with rituximab, n=2). After a central pathological review of 118 cases, grade 1 FL was diagnosed in 63 and grade 2 in 53 patients respectively. Of the 2 remaining patients, 1 had *in situ* FL and the other composite lymphoma (FL and lymphoplamocytoid). Pre-treatment characteristics are shown in Table 1.

A total of 693 cycles of chemotherapy were administered. Eight patients did not complete the 6 cycles. Of these, 2 patients developed disease progression after the first cycle, and in 2 patients the treatment was stopped after 2 cycles due to toxicity (progressive multifocal leukoencephalopathy, n=1; myelotoxicity, n=1). Two patients received 3 cycles after which treatment was stopped due to myelotoxicity (n=1) and disease progression (n=1). One patient died due to aspiration pneumonia after the fourth cycle and treatment was stopped in another patient after 5 courses due to myelotoxicity. The actual dose administered to patients completing 6 cycles was 90-100% of the planned dose in 90% of patients; 75-90% in 8%; 50-75% in 1% and <50% of the planned dose in 1%. G-CSF was administered in 21% of cycles: 38 patients received G-CSF with at least one of the cycles. The median interval between cycles was 28 days (range 19-64), with 8% of the cycles being delayed. Delayed treatment became more frequent at later cycles.

Response

Interim response was assessed after 3 cycles in 101 patients and it was shown that 56 patients had achieved CR, 42 PR, and 3 had developed disease progression. After treatment, 119 patients who had received at least 3 cycles were assessable for response: 99 (83%) achieved CR and 13 PR (11%). Seven patients (6%) were considered non-responders due to disease progression (n=5), early death due to aspiration pneumonia (n=1) and progressive multifocal leukoencephalopathy (n=1). Achievement of CR was associated with non-bulky disease (p=0.045), <2 extranodal sites (p=0.05), and no PB involvement (p=0.03). In logistic regression analysis, non-bulky disease (p=0.01; relative risk [RR]: 4.4) and no PB involvement (p=0.04; RR: 7.4) retained prognostic value for achievement of CR. Neither the FLIPI nor the IPI predicted for CR.

Twenty-two patients received additional treatment

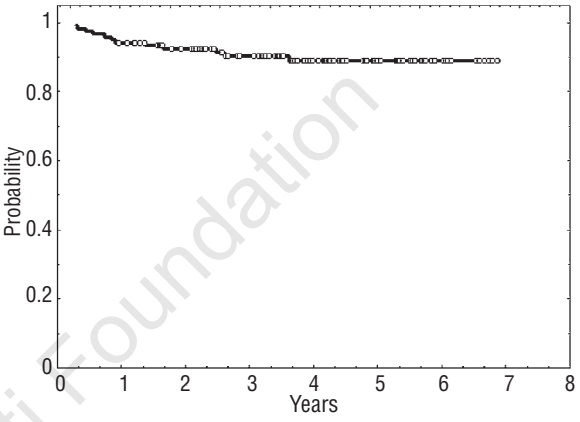

Figure 3. Overall survival in 120 patients with follicular lymphoma treated with the FCM regimen.

Table 2. Grade 3-4 hematologic toxicity after each cycle*.

FCM cycle	#1 n=112	#2 n=103	#3 n=108	#4 n=105	#5 n=101	#6 n=90	Total n=619
Neutrophil count	5	6	3	11	11	4	40 (6.4%)
Platelet count	2	0	1	2	1	1	7 (1.1%)
Hemoglobin	1	2	1	0	0	1	5 (0.8%)

**Blood tests were performed the day the following cycle was scheduled. N: n. of cycles with assessable data; neutrophil toxicity grade 3: 0.5-0.9×10⁹/L; neutrophil toxicity grade 4: <0.5×10⁹/L; platelet toxicity grade 3: 25-49×10⁹/L; platelet toxicity grade 4: <25×10⁹/L; hemoglobin toxicity grade 3: 65-79 g/L; hemoglobin toxicity grade 4: <65 g/L.*

Table 3. Hematologic counts after FCM therapy.

	6 months after FCM			12 months after FCM		
	N	Median	Range	N	Median	Range
Leukocyte*	58	3.5	1.8-9.7	51	4	1.9-18.2
Neutrophil*	58	2.1	0.8-6.4	51	2.4	0.8-14.7
Lymphocyte*	47	0.8	0.3-2.5	43	0.9	0.4-1.9
Hemoglobin#	58	12.9	9.6-14.1	51	13.3	10.7-16.4
Platelet*	58	162	39-272	51	163	73-295

*N: n. of patients with assessable data; *×10⁹/L; #g/L.*

after FCM. Fourteen were in CR (5 received rituximab; 5 IFN-α; 3 rituximab and IFN-α; and 1 Yttrium-90-ibritumomab tiuxetan) and the remainder in PR. Of those patients in PR, 6 achieved CR after treatment with Yttrium-90-ibritumomab tiuxetan (n=2), rituximab (n=2), rituximab and IFN-α (n=1) and rituximab and local radiotherapy (n=1). One patient remained in PR after rituximab and 1 after 3 further cycles of FCM. Additional therapy did not significantly change the final outcome of the patients in terms of RD.

BCL2/JH assessment and molecular follow-up

BCL2/JH breakpoints were assessed at diagnosis in 113 cases, with the following distribution: *MBR*, 64 (57%); *mcr*, 6 (5%); no *BCL2/JH* rearrangement detected, 43 (38%). Among the latter, paraffin samples were analyzed confirming the lack of rearrangement in 17 patients.Forty-six patients with rearrangement at *MBR* had an assessable PB sample both at diagnosis and after completing treatment. Thirty-seven of them (34 in clinical CR and 3 in PR) (81%) achieved an MR after FCM. Eight out of 9 patients without MR were in CR and 1 in PR. One year after treatment, PCR samples were available in 30 patients, 20 of them remaining negative.

Progression-free survival and response duration

Treatment failed in 39 patients. Five patients progressed while on treatment, there were 2 deaths during treatment, and 32 relapsed/progressed after achieving CR/PR. PFS at 5 years was 59% (95% confidence interval (CI): 49-69%). When patients who received some additional therapy were excluded from the analysis this figure was 58% (95% CI: 47-69%) (Figure 1). Variables associated with a shorter PFS were a poor PS (ECOG ≥2) (p=0.005), ≥2 extranodal sites (p=0.01), and high β2-microglobulin (β2-m) (p=0.03).

After a median follow-up of 3.9 years (range: 1-6.9 years), 32 patients in CR/PR after FCM relapsed and the 5-year RD was 63% (95%CI: 52-74%). The 5-year RD for patients who received additional treatment was 70% compared with 63% for the remainder (p>0.1). The risk of relapse/progression was higher for patients with ≥2 extranodal sites (p=0.03) and high/intermediate- or high-risk IPI (p=0.02). The degree of clinical response (CR *vs.* PR) did not predict RD. Achievement of an MR after treatment predicted RD (5-year RD: 78% *vs.* 48% for patients not achieving an MR; p=0.03), (Figure 2), although problems of data availability meant that a satisfactory multivariate model to test for the independence of this variable could not be obtained.

Overall survival

Twelve patients have died. Two died while receiving treatment. One of these, with a CD4 count of <0.3×10^9/L before FCM, was diagnosed with PML. Diagnosis was based on PCR for JC virus in the CSF and a brain biopsy immediately after the second cycle of FCM. The other died of aspiration pneumonia. Nine patients died after relapse due to progression (n=7) or toxicity of the salvage therapy (n=2). The remaining patient died in CR after brain surgery for meningioma. OS at 5 years was 89% (95%CI: 83-95%) (Figure 3). Poor PS (ECOG≥2) (p<0.0001) and high β2-m (p=0.001) predicted a shorter OS.

Early and late toxicity

Hematologic toxicity is shown in Table 2. Red cells and platelet transfusions were administered in 2.4% and 0.2% of the cycles respectively. Grade 3-4 infections were diagnosed in 2.4% of cycles, including neutropenic fever (n=13), respiratory infection (n=1), pneumonia (n=1) and PML (n=1). Other minor infections were seen in 33 cycles, including herpes zoster in 2 cases. Hematologic results at 6 and 12 months after completing the treatment are shown in Table 3. One patient was diagnosed with Hodgkin's lymphoma 46 months after completing treatment. No other late toxicity was observed.

Peripheral blood stem cell harvesting

Peripheral blood stem cell collection was attempted in 28 patients, 24 in response after FCM and 4 after a subsequent relapse and further treatment. Of the 24 in response, the median time from the last course of FCM to PBSC collection was 177 days (range 51-1296). The mobilization regimen at first attempt was G-CSF alone in 15 patients and cyclophosphamide with G-CSF in 9. In 18 patients, the first attempt at mobilization was unsuccessful (CD34$^+$cell count <2×10^6/kg) (13/15, 87%, after G-CSF mobilization; 5/9, 56%, after cyclophosphamide and G-CSF). The median time from the end of chemotherapy to mobilization was 127 days (range 51-417) in patients obtaining a CD34$^+$cell count <2×10^6/kg, compared with 245 days (range 127-1,296) in those achieving a CD34$^+$cell count >2×10^6/kg. Eight patients had a second PBSC collection procedure, a CD34$^+$cell count ≥2×10^6/kg was achieved in 2 patients and 1 patient had a third, unsuccessful, procedure. In total, PBSC collection was unsuccessful after 1-3 procedures in 16 patients (67%). In addition, 4 patients who subsequently relapsed had PBSC collected after salvage therapy, all of them after G-CSF mobilization.

Discussion

The results of the present study show the achievement of a high CR rate with prolonged PFS with FCM as upfront therapy for FL patients. Comparable results have been reported with similar combinations yielding a 5-year PFS ranging from 39%[23] to 53%.[24] It is interesting that all the patients in the Tsimberidou *et al.* study[23] received maintenance therapy with IFN-α whereas in the

current study the 5-year DR for 90 patients who did not receive additional treatment after FCM was 63%. Comparisons of different studies are problematic because of the different patient characteristics. Our patients were younger and there was a smaller percentage of them with B-symptoms, high LDH or high-risk FLIPI, although distribution according to the IPI was similar. However, our results compare favorably with recent reports including rituximab as part of front-line treatment.[25,26] This is important because treatment of patients with FL currently favors the use of rituximab-based combinations (e.g. CVP-R, CHOP-R, FCM-R).[25-27] However, the best partners for rituximab in the treatment of FL have not yet been identified. The fact that FCM yields a similar, if not better, response rate than some rituximab-based combinations is, in our opinion, of the utmost importance because it is logical to expect that FCM combined with rituximab could obtain even better results. In the current study, the disappearance of the *BCL2/JH* rearrangement was detected after treatment in 81% of patients and was associated with longer RD. Other reports have also shown a high MR rate in patients treated with fludarabine combinations.[28] It is interesting that absence of MRD after treatment correlates with good prognosis in patients treated with conventional chemotherapy,[5] high-dose chemotherapy[6] and rituximab.[8] The excellent results reported here were achieved with remarkably mild toxicity. Grade 3-4 neutropenia was seen in 6.4 of cycles, which translated into 16 episodes of grade 3-4 infection. This minor hematologic toxicity in comparison with that reported in other series might be related to the fact that blood counts are frequently reported at nadir. Also, in the current study, G-CSF was used more frequently than recommended in the protocol. Only 3 cases of minor viral infection were detected. This contrasts with the infection rate found with FND which might be related to the inclusion of dexamethasone.[29] No cases of PCP were diagnosed, which supports the importance of PCP prophylaxis,[30,31] even though some recent reports have suggested it might not be required.[32] The only serious viral complication was the diagnosis of PML in one patient after the second cycle of FCM. However, the CD4 count at diagnosis was 0.18×10^9/L, making any causative effect of FCM unlikely.

In spite of the almost negligible short-term myelotoxicity of FCM, the long-term effect on the BM is a matter of concern. In the present series, PBSC mobilization after treatment was unsuccessful in 16 out of 24 patients. Mobilization was more frequently successful when cyclophosphamide and G-CSF were used as the mobilization regimen than G-CSF alone. Also, there was a trend for a higher probability of successful harvesting when the median time from last chemotherapy to mobilization was longer, although the numbers are too small to draw any significant conclusions. The difficulty in obtaining PBSC after treatment with fludarabine-containing regimens has been previously reported.[31,33,34] The potentially harmful effects of prolonged immunosuppression caused by purine analogs must also be considered. Cheson *et al.*[35] analyzed the risk of secondary neoplasms in a large series, including patients refractory to alkylators, treated with purine analogues as a single agent for hairy cell leukemia or CLL and concluded that the use of purine analogues was not associated with a higher risk of secondary malignancies. However, in the CALGB randomized study comparing fludarabine, chlorambucil and the combination of both agents as first-line treatment for CLL, among 6 patients who developed treatment-related myelodysplastic syndromes or acute myeloid leukemia (t-MDS/t-AML), 5 had received both drugs.[36] Similarly, in a recent study reporting an increased risk of tMDS/AML following fludarabine combinations, 9 out of 10 patients who developed such a complication had previously received at least one line of treatment including alkylating agents.[37] By contrast, no t-MDS/t-AML have been reported with fludarabine and cyclophosphamide combinations as front-line treatment,[24] and this has been attributed to the lower leukemogenic potential of cyclophosphamide. In line with this, after a median follow-up of 47 months, no cases of t-MDS/t-AML have been observed in this series. Interestingly, this median follow-up is longer than the median time to t-MDS/t-AML (32 and 34 months) in patients receiving fludarabine combinations in two studies.[36,38] Prior therapy with fludarabine has been related to an increased risk of t-MDS/t-AML in patients subsequently treated with high-dose radio-chemotherapy with stem cell rescue[39] or with radio-immunotherapy.[40] Therefore, although the risk of t-MDS/t-AML with FCM as front-line therapy does not appear to be substantially increased, the impact of FCM on the risk of subsequent carcinogenic treatments remains a concern. Given this, it might be wise to avoid TBI-conditioning regimens or radio-immunotherapy in patients previously treated with FCM. In conclusion, FCM results in high CR and MR rates with prolonged RD without heavy toxicity, comparable to that achieved with some rituximab-based combination therapies. These results provide a strong basis for further studies to investigate the effectiveness of FCM with rituximab as front-line therapy in patients with FL.

Authorship and Disclosures

SM participated in the collection of the data, statistical analysis, interpretation of results and wrote the paper. CM and DC performed the PCR analysis. EDD, CE, AO, AA, JB, CP, SG, LE, AA, PV, PG, AFS, JMR and JB participated in the collection of the data and interpretation of results. EC performed the central pathological review. EM participated in the conception and design of the study and manuscript writing. ALG participated in the conception and design of the study, statistical analysis, interpretation of results and manuscript writing. All authors approved the final version of this paper. The authors reported no potential conflicts of interest.

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