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ORIGINAL ARTICLE

Phase II fludarabine and cyclophosphamide for the treatment of indolent B cell non-follicular lymphomas: final results of the LL02 trial of the Gruppo Italiano per lo Studio dei Linfomi (GISL)

Andrea Ferrario · Francesco Merli · Stefano Luminari · Caterina Stelitano · Donato Mannina · Mario Russo · Patrizio Mazza · Luigi Marcheselli · Maria Cecilia Goldaniga · Massimo Federico · Luca Baldini · for the Gruppo Italiano per lo Studio dei Linfomi

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Abstract Indolent non-follicular non-Hodgkin lymphomas (INFL) are a heterogenous subset whose treatment has been poorly investigated. In this context we have evaluated the efficacy and safety of combined fludarabine and cyclophosphamide (FC) upfront therapy. Sixty-three patients with advanced INFL were enrolled in the study. Therapy consisted in FC combination (25 and 250 mg/m^2, i.v., respectively, for three consecutive days) every 28 days for six courses. After histological review, 61 patients (36 men, median age 64 years, range 40–70 years) were evaluated (22 small lymphocytic, 11 lymphoplasmacytic, 25 marginal zone and 3 CD5-negative non-Hodgkin lymphomas not otherwise specified). Further two patients were excluded for lack of essential data; six patients were withdrawn before the third cycle because of WHO grade III and IV toxicity. At the final evaluation, the overall response rate was 83% with 40.7% of complete remission. Intention-to-treat analysis showed that at the median follow-up of 36 months, overall survival, progression-free survival and failure-free survival were respectively 78%, 60% and 46%; remission duration among the 49 patients achieving complete remission/partial remission at the end of treatment was 65% (44–78) without significant differences between the main histotypes. The most frequent grade III and IV toxic events were haematological (neutropaenia 34%, anaemia 18% and thrombocytopaenia 11%) and infectious (10%). FC is effective for advanced untreated INFL. Early deaths and haematological toxicity suggest careful patient selection and monitoring.

A complete list of the GISL centres referring patients for the study is given in a footnote at the end of this article.

A. Ferrario · M. C. Goldaniga · L. Baldini (✉)
Unità Operativa Ematologia 1, Fondazione IRCCS, Cà Granda, Ospedale Maggiore Policlinico, Dipartimento Scienze Mediche, Università degli Studi,
Via F. Sforza 35,
20122 Milan, Italy
e-mail: luca.baldini@unimi.it

F. Merli
Servizio di Ematologia, Arcispedale S. Maria Nuova, Reggio Emilia, Italy

S. Luminari · L. Marcheselli · M. Federico
Dipartimento di Oncologia ed Ematologia, Università di Modena e Reggio Emilia, Modena, Italy

C. Stelitano
Dipartimento di Ematologia, Azienda Ospedaliera Bianchi-Melacrino-Morelli, Reggio Calabria, Italy

D. Mannina
Divisione di Ematologia, Azienda Ospedaliera "Papardo", Messina, Italy

M. Russo
Ematologia e Immunoematologia, Ospedale S. Vincenzo, Taormina, Italy

P. Mazza
Divisione di Ematologia, Ospedale Moscati, Taranto, Italy

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Introduction

Indolent non-follicular non-Hodgkin B cell lymphomas (INFL) are clonal mature B cell proliferations that often present in leukaemic phase and include chronic lymphocytic leukaemia/small lymphocytic lymphomas (CLL/SLLs), lymphoplasmacytic lymphomas (LPCs) and marginal zone lymphomas (MZLs), a heterogeneous group with different presenting features, behaviour patterns and clinical outcomes [1]. As their clinicopathological presentation frequently makes a histological diagnosis difficult, this subset of lymphomas has been relatively less investigated, and only retrospective studies or prospective trials involving a limited series of patients have so far been published [2–9].

In 2003, the Italian Lymphoma Study Group (GISL) published the results of the LL01 trial [10] in which treatment-naive patients with advanced INFL were randomised to receive chlorambucil or chlorambucil plus epirubicin. The conclusion of this study underlined the fact that the addition of an anthracycline does not improve the overall response rate (ORR) and the survival in this subset of non-Hodgkin lymphomas (NHL). Since then, the purine analogue fludarabine has been considered one of the most active agents for the treatment of CLL and indolent NHLs; a number of studies have shown that it is superior to chlorambucil- [11] and anthracycline-containing regimens [12, 13] in terms of the number of complete remissions (CRs) and progression-free survival (PFS) in the first- and second-line treatment of CLL. The efficacy of fludarabine may be increased by combining it with other agents, and it has been observed that it has synergistic biochemical modulating in vitro effects on cyclophosphamide [14]. The combination of fludarabine and cyclophosphamide is the most widely investigated [15].

On the basis of these considerations and its previous experience with LL01, GISL started its LL02 multicentre prospective phase 2 trial in 2002 with the aim of evaluating the efficacy and safety of combined fludarabine and cyclophosphamide (FC) in the first-line therapy of INFL patients and verifying long-term outcomes.

Materials and methods

Patients

This multicentre, single-arm, phase II open-label GISL trial was conducted in 24 Italian haematology departments between July 2002 and September 2006 and enrolled 63 adult patients with documented advanced INFL. The inclusion criteria were a histological (pathological tissue and/or bone marrow) diagnosis of SLL, LPC or MZL made on the basis of the findings of a nodal or extranodal non-gastric biopsy in accordance with WHO criteria [1]; a diagnosis of SLL or LPC/Waldenstrom's macroglobulinaemia could be based on a bone marrow biopsy by testing immunoreactivity for CD20, CD5, CD23, CD10 and cyclin D1 only if no pathological tissue specimen was available. All of the histological samples were centrally reviewed by a pool of pathologists.

The other inclusion criteria were an age of 18–70 years; no previous lymphoma therapy; an Eastern Cooperative Oncology Group (ECOG) performance status of ≤3; Ann Arbor stage II (with more than three involved sites), III or IV; the presence of active disease defined by at least one of systemic B symptoms, bulky disease (maximum diameter >5 cm) or a doubling of the maximum diameter in at least three measurable sites in <12 months; haemoglobin (Hb) levels of <10 g/dL (due to lymphoma) and/or platelets (PLT) <100,000/μL (due to lymphoma); diffuse bone marrow infiltrate; or a lymphocyte doubling time of <12 months in leukaemic cases.

The exclusion criteria were: typical CLL, Richter's syndrome, mantle cell lymphoma (MCL), MALT gastric lymphoma, prolymphocytic leukaemia, hairy cell leukaemia, leukaemic $CD5^+$ lymphoproliferative disease; previous lymphoma treatment; pregnancy or lactation; a history of other malignancies (except for adequately treated carcinoma in situ) in the 3 months preceding study entry; heart failure (NYHA grades III and IV) or myocardial infarction in the 6 months preceding study entry; HIV seropositivity, active HBV and/or HCV infection; central nervous system (CNS) involvement of the lymphoma or epilepsy, CNS diseases and major psychiatric diseases; autoimmune cytopaenia (haemolytic anaemia, thrombocytopaenia) and Coombs' test positivity; creatinine more than twice the upper normal limit (UNL, >2.0 mg/dL, 177 mmol/L, unrelated to lymphoma), conjugated bilirubin more than twice the UNL (>2 mg/dL, 35 mmol/L), alkaline phosphatase and transaminases >2.5 times the UNL.

The protocol was approved by the GISL central Institutional Review Board and shared by all of the participating centres. All of the patients were required to sign an informed consent form before being enrolled in accordance with the Declaration of Helsinki. Accrual began July 2002, and so the study is not registered in any clinical trial registry. The patients were registered using a dedicated secure Web site, and the electronic case report forms were reviewed for accuracy by the local investigators.

Patients underwent pretreatment screening with baseline laboratory and imaging studies (CT or MRI), a pathological tissue biopsy if possible, bone marrow aspirate and biopsy.

Peripheral blood flow cytometry was used in leukaemic cases (lymphocytes≥5,000/μL). The follow-up evaluations were performed at months+3 and +6, and then six-monthly for 3 years, and annually thereafter. The interim analysis of the first ten enrolled patients (made in November 2003) showed that two patients aged more than 65 years with splenic MZL died during the initial phases of the treatment: the first due to post-splenectomy sepsis and the second due to post-aplasia pulmonary aspergillosis. Therefore, we introduced systematically the use of granulocyte colony-stimulating factor and antibiotic prophylaxis with levofloxacin in patients aged more than 65 years old.

Study design

The purpose of the study was to evaluate the efficacy and safety of FC in patients with advanced and untreated INFL aged <70 years. The primary end points were response rate and PFS. The secondary end points were the safety and tolerability of FC, overall survival (OS), and the suitability and efficacy of the inclusion criteria in distinguishing different clinical entities.

Treatment schedule

Patients fulfilling the inclusion criteria were intravenously treated with fludarabine 25 mg/m^2 plus cyclophosphamide 250 mg/m^2 daily for 3 days, repeated every 28 days for six courses. An intermediate evaluation was performed after three cycles: the patients in partial remission (PR) or CR received a further three FC courses, followed by radiation therapy for bulky disease if necessary; the patients with stable disease (SD) or progressive disease (PD) within a year of induction therapy were administered the salvage therapy in use in each centre. From day+7 of the first cycle to day+60 after the last dose of chemotherapy or the normalisation of the CD4$^+$ leucocyte count, the patients received oral thrimethoprim–sulphametoxazole twice a day twice weekly as prophylaxis against *Pneumocystis jiroveci* and fluconazole 200 mg daily as antimycotic prophylaxis. The use of therapeutic antibiotics, antiemetics, blood product transfusions and colony growth factor support was at the discretion of the attending physician. Final radiotherapy was permitted. The laboratory, toxicity and treatment delivery data entered in the GISL trial office data processing system are available at the Web site http://www.trialmanager.eu.

Response criteria

The patients were followed up until lymphoma progression or death. Modified Cheson response criteria were used [16], and a response had to be confirmed at two consecutive medical examinations separated by a 3-month interval. A CR was defined as the absence of constitutional symptoms or lymphoadenopathy, splenomegaly and hepatomegaly at physical and CT examination, a normal peripheral blood count and bone marrow biopsy findings, and the disappearance of a measurable M band. A PR was defined as a reduction of at least 50% in the size of measurable lesions, as measured by the sum of the products of the perpendicular diameters of the largest lesions, without the appearance of any new disease manifestations, and a reduction of at least 50% in the serum M band and the pretreatment number of peripheral blood lymphocytes for leukaemic cases (in the presence of a peripheral neutrophil count of >1,500/μL, a platelet count of >100,000/μL and a Hb level of >11 g/dL). SD was defined as a regression of <50% in the size of assessable disease sites or an increase of <25% in the size of existing lesions, without the appearance of any new disease manifestations, and PD as an increase of at least 25% in a site involved before therapy or the appearance of any new disease manifestations.

Statistical analysis

This was a multicentre, open-label, non-comparative phase II study. A total of 63 patients entered the protocol, and the intent-to-treat population included 61 patients with revised histologic diagnosis. OS was defined as the time from the date of study entry to the date of last observation or death due to any cause. Failure-free survival (FFS) was defined as the time from the date of study entry to the last follow-up visit, or to one of the following events: any response less than PR at the end of therapy, documented progression, and relapse or death due to any cause other than lymphoma. PFS was measured from the date of study entry to the last follow-up visit or disease progression during treatment, relapse or death due to any cause. The duration of remission was defined as the time from the date of CR or PR after therapy to the date of disease progression, or the date of the last follow-up examination. Survival functions were estimated on the basis of an intention-to-treat analysis using the Kaplan–Meier method [17]. Toxicity was recorded and graded using a four-point scale according to the ECOG criteria [18].

Results

Patient flow

Between July 2002 and September 2006, 63 adult patients with INFL were consecutively enrolled. After registration, two cases were excluded because of a revised histological diagnosis (one T cell-rich B cell

lymphoma and one multiple myeloma with nodal involvement). Table 1 shows the main clinical and haematological characteristics of the remaining 61 patients (36 men and 25 women, M/F 1.44; median age 64 years, range 40–70 years). The diagnoses were made on nodal specimens (39%), extranodal pathological samples (12%) and bone marrow biopsy (49%). There were 22 cases of SLL, 11 of LPC, 25 of MZL (6 nodal, 6 non-gastric extranodal and 13 splenic), and 3 of CD5-negative low-grade B cell NHL. Twenty patients presented a peripheral clonal B cell population.

Two patients were subsequently excluded because no further information was obtained after registration. During the study, 6 of the 59 evaluable patients (10%) were withdrawn from the study before treatment completion: two splenic MZL cases because of fatal infectious episodes (one pulmonary aspergillosis after the second cycle and one bacterial pneumonia after the third cycle); three because of WHO grade III and IV haematological toxicities (two prolonged pancytopaenia after the first (SLL case) and one (MZL) after the second cycle and a haemolytic anaemia in one case of MZL after the first cycle); and one MZL because of WHO grade IV renal toxicity after the first cycle. Of the 53 remaining patients, 43 completed the planned treatment of six cycles. Three stopped treatment after five cycles: one SLL and one MZL because of WHO grade III prolonged pancytopaenia and one SLL because of skin infection in the presence of WHO grade III neutropaenia. Four stopped after four cycles (one SLL, one LPC and one MZL because of WHO grade III prolonged pancytopaenia and one MZL because of WHO grade III anaemia), two after three cycles (one CD5-negative low-grade B cell NHL because of grade III neutropaenia and one MZL because of WHO grade III renal toxicity), and one patient progressed after the first cycle developing a haemolytic anaemia (Fig. 1).

Table 1 Clinico-haematological baseline characteristics of assessable patients (N=61)

Characteristic		Number	Percentage
Age (years)	<65	33	54
	≥65	28	46
Sex	Male	36	59
	Female	25	41
Ann Arbor stage	II	1	2
	III	2	3
	IV	58	95
B symptoms	Present	13	21
	Absent	48	79
Bulky	Negative	41	67
	Positive	20	33
Serum LDH	≤UNL	41	67
	>UNL	20	33
Serum β_2-microglobulin (n=51)	≤UNL	14	27
	>UNL	37	73
Splenic involvement	Negative	29	48
	Positive	11	18
	Splenomegaly	21	34
Hb	≤11 g/dL	42	69
	>11 g/dL	19	31
PLT	≤100,000/μL	49	80
	>100,000/μL	12	20
Lymphocytosis	≥5,000/μL	20	33
	<5,000/μL	41	67
Serum monoclonal component	Absent	19	31
	Present	42	69
Histologic diagnosis:			
Small lymphocytic lymphoma		22	36
Lymphoplasmacytic lymphoma		11	18
Marginal zone lymphoma		25	41
CD5 negative low-grade B cell NHL		3	5

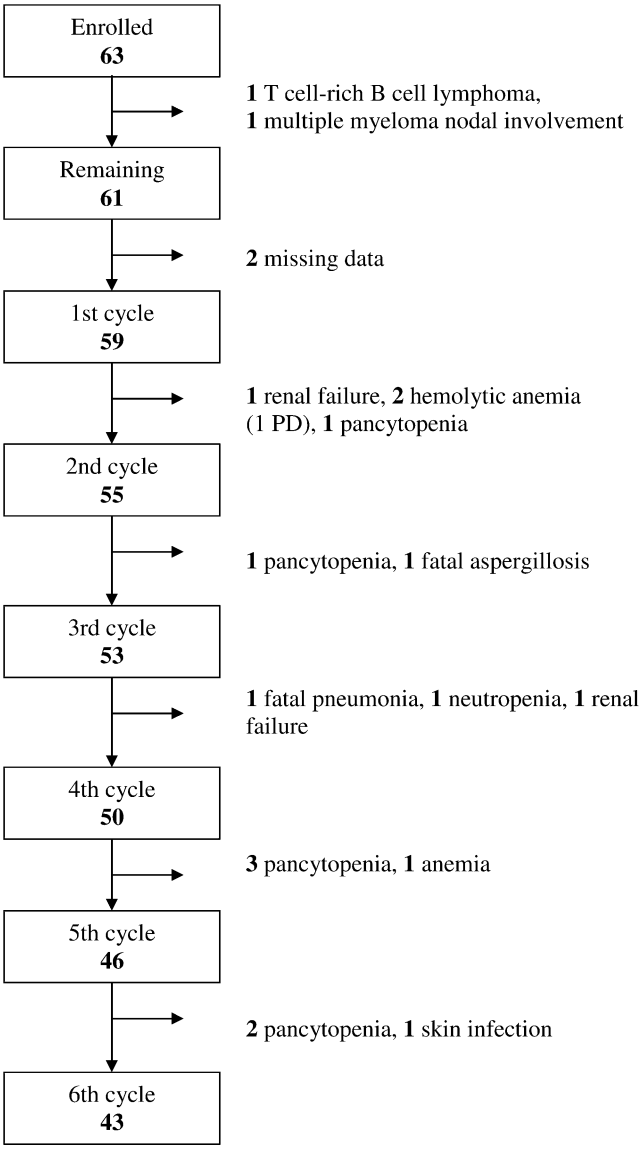

Fig. 1 Flowchart of the current study. *PD* progressive disease

Response to treatment and survival

After a median follow-up of 36 months, 17 patients had died (28%): six because of disease relapse/progression (10%), five because of sepsis (8%), three because of a second tumour (5%) and three of other causes (5%, including one cerebrovascular event and one case of respiratory insufficiency). Of the patients who died because of a second tumour, one (a 72-year-old male) died of myelodysplastic syndrome 3 years after the fourth FC cycle, the second (a 60-year-old male) died of acute myeloid leukaemia (M6) 1 year after the sixth FC cycle, and the third (a 62-year-old male) died of metastatic lung carcinoma 3 years after the sixth FC cycle.

At the intermediate evaluation, 51 of 59 patients (86.4%) showed an objective response (ORR), with 22.0% CR and 64.4% PR; one achieved SD, two had PD and five were withdrawn from the study before treatment completion. At the final evaluation, 83% showed an ORR, with 40.6% CR (24 patients) and 42.4% PR (25 patients); three patients had PD (5.0%) and one SD (1.6%).

On the basis of an intention-to-treat analysis, at a median follow-up of 36 months, OS was 78% (95% CI 65–87), PFS was 60% (95% CI 44–73) and FFS was 46% (95% CI 32–58; Fig. 2). At the median follow-up, remission duration (RD) among the 49 patients achieving CR/PR at the end of treatment was 65% (44–78); in the subset of SLL and MZL patients, RD were 59% (29–79) and 65% (38–83), respectively (Fig. 3).

Toxicity

The main toxicity was haematological. Considering the occurrence of at least one episode of grade III or IV toxicity, 18% of the patients presented anaemia, 34% neutropaenia and 11% thrombocytopaenia; 10% of the patients experienced WHO grade III and IV infectious toxicity. Two early infectious deaths were related to severe haematological toxicity, which led to treatment discontinuation in seven patients.

Discussion

For a number of decades, the conventional therapy for low-grade NHL has been based on alkylating agents (chlorambucil or cyclophosphamide) administered in monotherapy or in combination with or without anthracyclines depending on clinical aggressiveness (cyclophosphamide, vincristine and prednisone or cyclophosphamide, vincris-

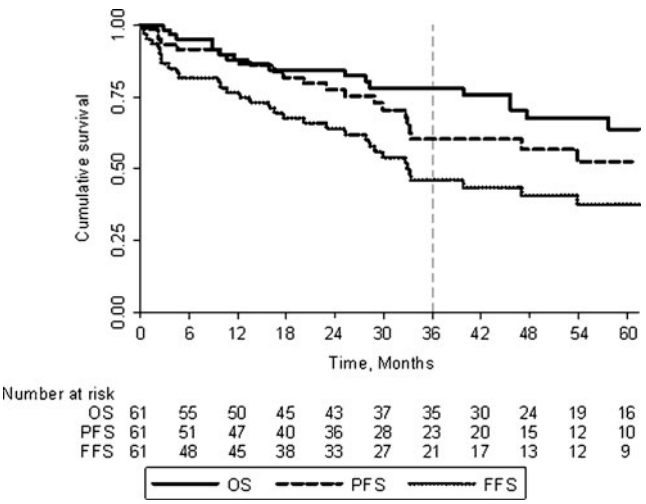

Fig. 2 Overall survival (*OS*), progression-free survival (*PFS*) and failure-free survival (*FFS*) of 61 eligible patients

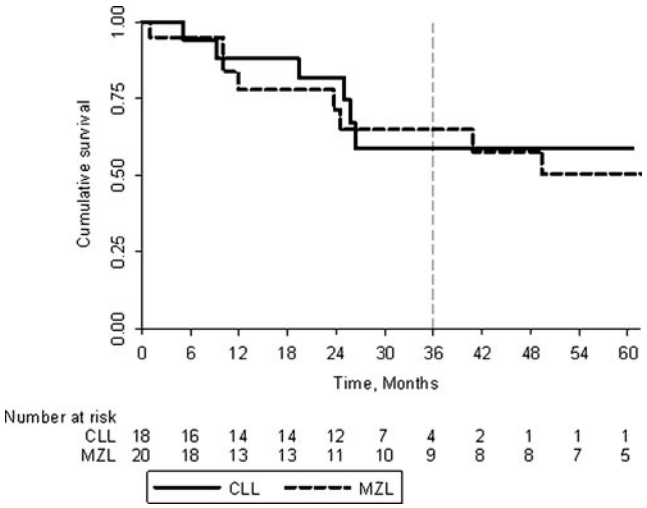

Fig. 3 RD of 49 patients in CR/PR at the end of treatment according to main histotypes

tine, prednisone and doxorubicin). The unsatisfactory results in terms of complete remission and long-term disease control induced investigating the role of purine analogues in this subset of diseases.

The fludarabine and cyclophosphamide combination has been extensively studied mainly in CLL. As first-line treatment, it is associated with high rates of OR (86–100%) and CR (20–60%) [15, 19, 20]. These results have been recently confirmed by three phase III studies comparing FC with fludarabine alone or chlorambucil [21–23].

However, its efficacy in other histological subtypes has been little investigated, and the results are generally based on subgroup analyses of mixed histological series [24]. Excellent OR and CR rates have been reported in untreated follicular lymphoma (90–100% and 60–89%) [25, 26] and MCL (80–100% and 40–70%) [24], although lower response rates have been described in patients with treated/untreated Waldenstrom's macroglobulinaemia (ORR 55%; CR 0%) [27]. The results of our study confirm the remarkable efficacy of FC, especially in terms of the number of CRs, a percentage similar to those found in other published studies [25], with the exception of the lower percentage described by Tam et al. [24] and the higher percentage described by Hochster et al. [26]. This last difference can be partially explained by the fact that this study included younger patients than ours (only 26% was older than 60 years) and a large majority of follicular NHLs. Ours is the first study that includes only non-follicular low-grade NHLs and exclude CLL, which makes it difficult to compare the results with those of other studies. Furthermore, the long follow-up of our series allows us to draw consistent data about survival parameters and long-term toxicities associated with FC combination in pre-rituximab era.

Another important aspect that complicates data interpretation is the heterogeneity of the published FC schedules: some authors used a single dose of cyclophosphamide added to 5 days of fludarabine [25, 26], whereas others used the daily co-administration of various doses of both drugs on the basis of the findings of preclinical studies [15, 24].

As in other published studies, we found that the main toxicity was haematological, which led to six patients (10%) stopping the therapy before the intermediate evaluation; moreover, further eight patients could not complete the programme because of myelosuppression. Grade IV neutropaenia associated with severe drug-related lymphocytopaenia caused a substantial rate of infectious complications: four patients died during a prolonged post-chemotherapy aplastic phase of uncontrolled sepsis (two because of disseminated aspergillosis and two because of bacterial septicaemia). This percentage of fatal infectious was higher than that reported in other studies, even though patients systematically received antimicrobial prophylaxis [24, 25]; interestingly, all these patients were affected by NHL of marginal zone (three splenic and one nodal) and three were older than 65 years. The most important complication after myelosuppression was the early appearance of second tumours during the follow-up period: two hematopoietic disorders and one solid tumour.

Therapy-related myelodysplasia and acute myeloid leukaemia (tMDS/tAML) are well-recognised complications of fludarabine and alkylating agent combination regimens [28], and up to 10% of lymphoma patients treated with standard chemotherapy may develop tMDS/tAML [29].

The combination of fludarabine with cyclophosphamide may increase the risk of tMDS/tAML probably because of the synergistic effects in the induction and inhibition of DNA repair following DNA damage [30].

In conclusion, FC chemotherapy is effective in advanced untreated non-follicular low-grade NHLs and leads to very good ORR, CR and PFS rates. The crucial point remains OS, which does not seem to be significantly improved in comparison with fludarabine alone or standard therapy in other series including different histotypes of low-grade NHLs. In our study, the OS was conditioned by haematological and infectious toxicities that caused toxic deaths and the discontinuation of the planned treatment in a significant subset of patients; the role of this combination in inducing the development of second tumours may also have affected OS, although their relationship needs to be investigated in a larger number of patients. All of these data suggest that the use of this active combination should be reserved for carefully selected patients who need to be closely monitored during therapy. These results could be considered as the basis for the development of immunochemotherapy association, such as rituximab (R) FC (FCR), which has

been demonstrated to be an effective treatment for newly diagnosed as well as relapsed low-grade NHL [31, 32]. More recently, in two randomised trial in CLL at diagnosis and previously treated, FCR has been demonstrated to be more effective than FC chemotherapy [33, 34]. We have recently reported the preliminary results of this combination in this subset of lymphomas [35].

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The authors have declared no conflicts of interest.

References

1. Jaffe ES, Harris NL, Stein H, Vardiman JW (2001) World Health Organization classification of tumours of hematopoietic and lymphoid tissues, 3rd edn. IARC, Lyon
2. Ben-Ezra J, Burke JS, Swartz WG, Brownell MD, Brynes RK, Hill LR et al (1989) Small lymphocytic lymphoma: a clinicopathologic analysis of 268 cases. Blood 73:579–587
3. Coiffier B, Thieblemont C, Felman P, Salles G, Berger F (1999) Indolent non follicular lymphomas: characteristics, treatment, and outcome. Semin Hematol 36:198–208
4. Landgren O, Tilly H (2008) Epidemiology, pathology and treatment of non-follicular indolent lymphomas. Leuk Lymphoma 49(Suppl 1):35–42
5. Pangalis GA, Angelopoulou MK, Vassilakopoulos TP, Siakantaris MP, Kittas C (1999) B-chronic lymphocytic leukemia, small lymphocytic lymphoma, and lymphoplasmacytic lymphoma, including Waldenstrom's macroglobulinemia: a clinical, morphologic, and biologic spectrum of similar disorders. Semin Hematol 36:104–114
6. Papamichael D, Norton AJ, Foran JM, Mulatero C, Mathews J, Amess JA et al (1999) Immunocytoma: a retrospective analysis from St Bartholomew's Hospital—1972 to 1996. J Clin Oncol 17:2847–2853
7. Seng JE, Peterson BA (1997) Indolent B-cell non-Hodgkin's lymphomas. Oncology (Williston Park) 11:1883–1894
8. Treon SP (2009) How I treat Waldenström macroglobulinemia. Blood 17:2375–2385
9. Neparidze N, Dhodapkar MV (2009) Waldenstrom's macroglobulinemia: recent advances in biology and therapy. Clin Adv Hematol Oncol 7:677–681, 687–690
10. Baldini L, Brugiatelli M, Luminari S, Lombardo M, Merli F, Sacchi S et al (2003) Treatment of indolent B-cell nonfollicular lymphomas: final results of the LL01 randomized trial of the Gruppo Italiano per lo Studio dei Linfomi. J Clin Oncol 21:1459–1465
11. Rai KR, Peterson BL, Appelbaum FR, Kolitz J, Elias L, Shepherd L et al (2000) Fludarabine compared with chlorambucil as primary therapy for chronic lymphocytic leukemia. N Engl J Med 343:1750–1757
12. Johnson S, Smith AG, Loffler H, Osby E, Juliusson G, Emmerich B et al (1996) Multicentre prospective randomised trial of fludarabine versus cyclophosphamide, doxorubicin, and prednisone (CAP) for treatment of advanced-stage chronic lymphocytic leukaemia. The French Cooperative Group on CLL. Lancet 347:1432–1438
13. Leporrier M, Chevret S, Cazin B, Boudjerra N, Feugier P, Desablens B et al (2001) Randomized comparison of fludarabine, CAP, and ChOP in 938 previously untreated stage B and C chronic lymphocytic leukemia patients. Blood 98:2319–2325
14. Bellosillo B, Villamor N, Colomer D, Pons G, Montserrat E, Gil J (1999) In vitro evaluation of fludarabine in combination with cyclophosphamide and/or mitoxantrone in B-cell chronic lymphocytic leukemia. Blood 94:2836–2843
15. O'Brien SM, Kantarjian HM, Cortes J, Beran M, Koller CA, Giles FJ et al (2001) Results of the fludarabine and cyclophosphamide combination regimen in chronic lymphocytic leukemia. J Clin Oncol 19:1414–1420
16. Cheson BD, Horning SJ, Coiffier B, Shipp MA, Fisher RI, Connors JM et al (1999) Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. NCI Sponsored International Working Group. J Clin Oncol 17:1244–1253
17. Kaplan EI, Meier P (1958) Non parametric evaluation from incomplete observations. J Am Stat Assoc 53:457–481
18. Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET et al (1982) Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649–655
19. Hallek M, Schmitt B, Wilhelm M, Busch R, Krober A, Fostitsch HP et al (2001) Fludarabine plus cyclophosphamide is an efficient treatment for advanced chronic lymphocytic leukaemia (CLL): results of a phase II study of the German CLL Study Group. Br J Haematol 114:342–348
20. Schiavone EM, De SM, Palmieri S, Annunziata M, Pocali B, Copia C et al (2003) Fludarabine plus cyclophosphamide for the treatment of advanced chronic lymphocytic leukemia. Eur J Haematol 71:23–28
21. Catovsky D, Richards S, Matutes E, Oscier D, Dyer MJ, Bezares RF et al (2007) Assessment of fludarabine plus cyclophosphamide for patients with chronic lymphocytic leukaemia (the LRF CLL4 Trial): a randomised controlled trial. Lancet 370:230–239
22. Eichhorst BF, Busch R, Hopfinger G, Pasold R, Hensel M, Steinbrecher C et al (2006) Fludarabine plus cyclophosphamide versus fludarabine alone in first-line therapy of younger patients with chronic lymphocytic leukemia. Blood 107:885–891
23. Flinn IW, Neuberg DS, Grever MR, Dewald GW, Bennett JM, Paietta EM et al (2007) Phase III trial of fludarabine plus cyclophosphamide compared with fludarabine for patients with previously untreated chronic lymphocytic leukemia: US Intergroup Trial E2997. J Clin Oncol 25:793–798
24. Tam CS, Wolf MM, Januszewicz EH, Prince HM, Westerman D, Seymour JF (2004) Fludarabine and cyclophosphamide using an attenuated dose schedule is a highly effective regimen for patients with indolent lymphoid malignancies. Cancer 100:2181–2189
25. Flinn IW, Byrd JC, Morrison C, Jamison J, Diehl LF, Murphy T et al (2000) Fludarabine and cyclophosphamide with filgrastim support in patients with previously untreated indolent lymphoid malignancies. Blood 96:71–75
26. Hochster HS, Oken MM, Winter JN, Gordon LI, Raphael BG, Bennett JM et al (2000) Phase I study of fludarabine plus cyclophosphamide in patients with previously untreated low-grade lymphoma: results and long-term follow-up—a report from the Eastern Cooperative Oncology Group. J Clin Oncol 18:987–994
27. Dimopoulos MA, Hamilos G, Efstathiou E, Siapkaras I, Matsouka C, Gika D et al (2003) Treatment of Waldenstrom's macroglobulinemia with the combination of fludarabine and cyclophosphamide. Leuk Lymphoma 44:993–996
28. Tam CS, Seymour JF, Prince HM, Kenealy M, Wolf M, Januszewicz EH et al (2006) Treatment-related myelodysplasia following fludarabine combination chemotherapy. Haematologica 91:1546–1550

29. Armitage JO, Carbone PP, Connors JM, Levine A, Bennett JM, Kroll S (2003) Treatment-related myelodysplasia and acute leukemia in non-Hodgkin's lymphoma patients. J Clin Oncol 21:897–906
30. Yamauchi T, Nowak BJ, Keating MJ, Plunkett W (2001) DNA repair initiated in chronic lymphocytic leukemia lymphocytes by 4-hydroperoxycyclophosphamide is inhibited by fludarabine and clofarabine. Clin Cancer Res 7:3580–3589
31. Tam CS, O'Brien S, Wierda W, Kantarjian H, Wen S, Do KA et al (2008) Long-term results of the fludarabine, cyclophosphamide, and rituximab regimen as initial therapy of chronic lymphocytic leukemia. Blood 112:975–980
32. Tam CS, Wolf M, Prince HM, Januszewicz EH, Westerman D, Lin KI et al (2006) Fludarabine, cyclophosphamide, and rituximab for the treatment of patients with chronic lymphocytic leukemia or indolent non-Hodgkin lymphoma. Cancer 106:2412–2420
33. Hallek M, Fingerle-Rowson G, Fink AM, Busch R, Mayer J, Hensel M et al (2009) First-line treatment with fludarabine (F), cyclophosphamide (C), and rituximab (R) (FCR) improves overall survival (OS) in previously untreated patients (pts) with advanced chronic lymphocytic leukemia (CLL): results of a randomized phase III trial on behalf of an International Group of Investigators and the German CLL Study Group. Blood 114:535 (ASH Annual Meeting Abstracts)
34. Robak T, Dmoszynska A, Solal-Céligny P, Warzocha K, Loscertales J, Catalano J et al (2010) Rituximab plus fludarabine and cyclophosphamide prolongs progression-free survival compared with fludarabine and cyclophosphamide alone in previously treated chronic lymphocytic leukemia. J Clin Oncol 28:1756–1765
35. Baldini L, Pulsoni A, Rossi G, Vitolo U, Morra E, Olivero B, Cavalieri E, Merli F, Rigacci L, Nobile F, Ferrario A, Orsucci L, Brugiatelli M, Musto P, Lambertenghi Deliliers G, Gamba E (2009) Front-line chemo-immunotherapy rituximab-FC+rituximab maintenance in patients with untreated advanced stage non follicular lymphoma (INFL): update of a prospective study of the Intergruppo Italiano Linfomi and Roche (ML18324). Blood 114:2686 (ASH Annual Meeting Abstracts)

Other participating institutions include the following: Lombardo M., Oncologia Pescara; Bagnuolo A., Oncologia, Correggio; Nunzi M., Medicina e Oncologia, Terni; Mastrullo L., Ematologia, Ospedale S Gennaro, Napoli; Fregoni V., Oncologia, Istituto scientifico fondazione Maugeri, Pavia; Centurioni R,. Medicina e Ematologia, Civitanova Marche; Giglio G., Az. Osp Cardarelli, Campobasso; Ginaldi L., Università di l'Aquila-Teramo, Teramo, Italy; Riezzo A., Divisione di Ematologia, Trani; Fragasso A., Oncoematologia, Matera; Caparrotti G., Ematologia, Caserta; Partesotti G., Oncologia, Sassuolo; Vallisa D., Ematologia, Piacenza; Girmenia GP, Medicina e Ematologia, Abbiategrasso; Polimeno G., Medicina e Ematologia, Acquaviva delle Fonti; Musolino C., Ematologia, Policlinico, Messina.