

# Significant efficacy of 2-CdA with or without rituximab in the treatment of splenic marginal zone lymphoma (SMZL)

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**Background:** Splenic marginal zone lymphoma (SMZL) with or without villous lymphocytes is an indolent lymphoma that typically affects elderly patients. Treatment is required in symptomatic cases. Splenectomy remains one of the first-line options in patients fit for surgery. The best therapeutic strategy has not yet been identified. Among different possible chemotherapeutic approaches, purine analogues, alone or in association with rituximab, seem to be a valid therapeutic choice.

**Patients and methods:** Fifty SMZL patients were treated with cladribine with or without anti-CD20 mAb.

**Results:** Forty-six of 50 patients were assessable for response. Overall response rate was 87%: 24 of 46 patients (52%) achieved a complete hematological response (CR), 16 of 46 (35%) a partial response and 6 (13%) were unresponsive. Interestingly, 15 of 24 cases (62%) in CR also achieved a molecular remission.

**Conclusions:** The present results indicate that this schedule is a valid therapeutic approach in SMZL. Addition of rituximab significantly improved quality of response and consequently the outcome of the disease.

**Key words:** cladribine, rituximab, splenic marginal zone lymphoma

## Introduction

The term marginal zone lymphomas (MZL) comprises, according to the World Health Organization (WHO) classification, three distinct pathologic entities: splenic marginal zone lymphoma (SMZL), with or without villous lymphocytes, mucosa-associated lymphoid tissue type and nodal MZL (monocytoid), with or without monocytoid B cells [1, 2].

MZL are B-cell indolent lymphoproliferative disorders, considered to be relatively rare [5%–17% of all non-Hodgkin's lymphoma (NHL) in adults]. SMZL accounts for <2% of all NHL; it characteristically affects elderly or middle-aged patients and has a median survival of >10 years [3–6]. Malignant cells present features of mature, activated B lymphocytes, with expression of B-cell CD markers (CD19, CD20, CD22, CD79b and FMC7) with light-chain restriction. In the majority of cases, neoplastic lymphocytes are negative for CD5, CD23, CD10 and CD103. Bone marrow infiltration pattern is typically intrasinusoidal, but it can become nodular as the disease progresses or after splenectomy [7, 8].

SMZL characteristically presents massive splenomegaly, abdominal discomfort, lymphocytosis and cytopenias, often related to hypersplenism. Lymph node and/or organ

involvement are infrequent at the diagnosis but they may develop with progression of the disease. B symptoms are present in 25%–60% of cases, and autoimmune phenomena are not uncommon (15%–20% of patients). Serum paraproteinemia, usually <20 g/l, is observed in ~10% to 25% of patients. Association with hepatitis C virus (HCV) infection has been registered in South European countries, supporting a role of HCV in lymphomagenesis through chronic stimulation and indirect transformation of lymphoid cells. No specific prognostic factors have been established for SMZL: high tumor mass, hemoglobin (Hb) level <12 g/dl, increased lactate dehydrogenase (LDH), albumin <3.5 g/dl and alteration of  $\beta$ 2-microglobulin at diagnosis have been described as adverse prognostic factors [3, 6, 9, 10].

To date, standard criteria inducing the clinician to treat SMZL have not yet been defined. There is no clear advantage to the early therapy, which is considered as indicated when patients have developed significant signs and/or symptoms (severe cytopenias, symptomatic splenomegaly, recurrent infections and systemic symptoms). A 'watch and wait' policy is also reasonable for asymptomatic patients who have moderate cytopenias and nonbulky splenomegaly.

Therapeutic strategies comprise splenectomy and chemotherapy.

Alkylating agents, such as purine analogues, have been employed in these patients, with variable results [11, 12]. The therapeutic impact of cladribine (2-chlorodeoxyadenosine,

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2-CdA) has not yet been defined. Virchis et al. reported a high rate of good responses, whereas others described only partial remissions and frequent relapses [13, 14].

Our group previously reported a high rate of overall responses in cases receiving 2-CdA: 10 SMZL patients were treated with low-dose cladribine with very encouraging responses [overall response rate (ORR) 80%] [15].

Interestingly, in HCV-positive cases, the antiviral treatment appears able to induce lymphoma remission [16, 17]. Moreover, in recent years, the anti-CD20 antibody turned out to be a promising approach also in this histotype of NHL [18–21].

In the present study, we decided to retrospectively evaluate the activity of 2-chlorodeoxyadenosine with or without rituximab in 50 SMZL patients. Thirty-eight of 50 cases received anti-CD20 mAb: indeed, 15 cases received rituximab concomitantly with 2-CdA and other 17 after treatment, as consolidation.

Moreover, the prognostic impact of LDH,  $\beta$ 2-microglobulin, albumin and Hb levels was also evaluated in this series.

## patients and methods

Fifty patients affected by SMZL, with or without villous lymphocytes, entered into the study. Eligibility criteria were as follows: histological diagnosis of SMZL; age >18 years; life expectancy >6 months; absence of renal, hepatic and respiratory failure; no evidence of active infections; human immunodeficiency virus (HIV) negativity; two or more signs of active disease [symptomatic splenomegaly, constitutional symptoms (fever or night sweats) and severe peripheral cytopenias] and written informed consent.

Patient evaluation included a full history and clinical examination, complete serum biochemistry with dosage of LDH and  $\beta$ 2-microglobulin; peripheral blood and bone marrow immunophenotyping; bone marrow biopsy; bone marrow molecular analysis; chest and abdomen and pelvic computed tomographic scan and serology for HIV, hepatitis B virus and HCV.

Diagnosis was based on lymphocyte morphology, immunophenotype of peripheral blood and bone marrow samples, bone marrow biopsy and spleen histology when available.

Moreover, molecular study of IgH and BCL2/JH rearrangements was carried out by fluorescent PCR [22], to confirm the clonality of the disorder.

Patients' clinical characteristics are shown in Table 1. Ten of 50 patients had been previously treated (two with oral cyclophosphamide, four with chlorambucil, one with chlorambucil and oral cyclophosphamide, one with combination chemotherapy with cyclophosphamide, doxorubicin, vincristine and prednisone, one according to the fludarabine, idarubicin and steroid schedule and one with rituximab alone). Seven patients underwent splenectomy. Forty-four of 50 cases presented bone marrow involvement documented by biopsy (stage IV). Thirty-eight patients showed positivity for IgH rearrangement at molecular assay before treatment. Four cases were HCV positive.

### treatment

Cladribine was administered i.v. at a dose of 5 mg/m<sup>2</sup>/week for a total of six cycles. The rationale for choosing this schedule was based on the very good results obtained in hairy-cell leukaemia (HCL) patients treated with weekly administration of 2-CdA [23]. All patients received aciclovir, fluconazole

**Table 1.** Clinical characteristics of patients

Median age (years)	64 (33–85)
Sex (male : female)	24 : 26
Median LDH value (U/l)	386 (137–1478)
Median hemoglobin value (g/dl)	12.6 (8.5–16.4)
Median albumin value (mg/dl)	4 (3–5)
Median $\beta$ 2-microglobulin value ( $\mu$ g/l)	2900 (1261–6900)
Splenectomy (no. of patients)	7
Pre-treated patients	10
Oral cyclophosphamide	2
Chlorambucil	4
Oral cyclophosphamide and chlorambucil	1
CHOP	1
FLIDA	1
Rituximab	1

LDH, lactate dehydrogenase; CHOP, combination chemotherapy with cyclophosphamide, doxorubicin, vincristine and prednisone; FLIDA, fludarabine, idarubicin and steroid.

and co-trimoxazole as anti-infective prophylaxis until 8 weeks after completion of 2-CdA administration.

From 2000 to 2001, 18 patients did not receive rituximab; after that period, a further 32 cases were treated with antibody anti-CD20. In 17 of 32 cases (53%), rituximab was administered after chemotherapy for four doses; in the remaining 15 cases, the anti-CD20 mAb was infused concomitantly with cladribine for a total of six cycles. Rituximab was infused over a 3- to 6-h period, on an outpatient basis. Patients were premedicated with diphenhydramine (40 mg orally) and acetaminophen (1 g orally).

Patients were evaluated for response 2 months after the end of treatment, then every 3 months during the first 2 years and every 6 months for a further 3 years.

Response criteria were the following: (i) complete hematological response (CR) was defined as resolution of organomegaly, normalization of the blood count (Hb >12 g/dl, platelets >100  $\times$  10<sup>9</sup>/l, neutrophils >1.5  $\times$  10<sup>9</sup>/l and no evidence of circulating malignant B cells) and no evidence of bone marrow infiltration detected by immunohistochemistry; (ii) partial response (PR) was defined as  $\geq$ 50% improvement of all signs and symptoms of the disease (resolution or decrease of spleen size and of lymphadenopathies and improvement of cytopenias) and (iii) stable or progressive disease was computed as 'no response'.

WHO criteria (WHO, 1979) were used to assess toxicity.

### statistical analyses

All calculations were carried out using the SPSS for Windows, release 15, 2007. Overall survival and time to progression (TTP) were estimated using the Kaplan–Meier test. TTP was computed from the beginning of treatment to further disease progression, relapse or death.

## results

Fifty patients (24 male and 26 female; median age 64 years, range 33–81) affected by SMZL and treated from January 2000 to October 2008 at our institution were evaluated in the present retrospective study.

Overall, 46 patients were assessable for clinical response. ORR was 87%: 24 of these patients (52%) achieved a CR, 16 of

46 (35%) a PR and 6 (13%) were unresponsive. Interestingly, in the subgroup of 17 cases who underwent rituximab as consolidation, the CR rate after cladribine was 11%, but this percentage increased up to 66% after the anti-CD20 antibody.

Moreover, in the whole series, the quality of response was significantly influenced by the addition of rituximab: CR rate was 62.5% in the subgroup receiving the anti-CD20 antibody versus 21.4% for cases treated with cladribine alone ( $P = 0.004$ ). It should be noted that the schedule of rituximab administration (concomitant or after cladribine) did not condition the achievement of CR.

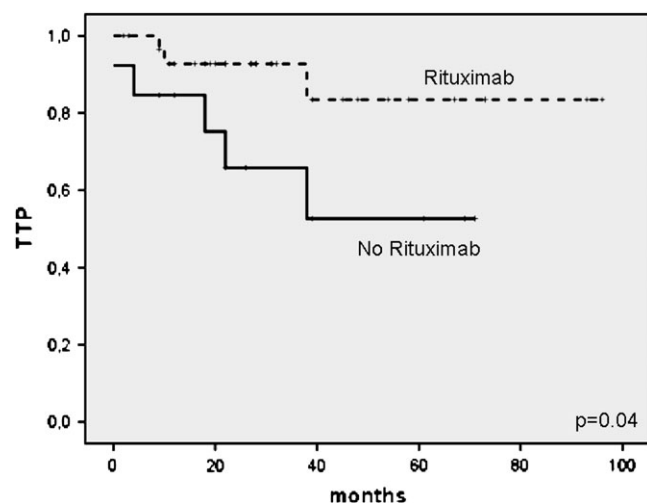
One patient died of disease progression after 63 months from the end of treatment and another one after 65 months due to gastric carcinoma, with a 6-year overall survival of the entire series of 89%.

With a median follow-up of 45 months (range 3–150), the 48-month TTP was 67%; it was not significantly influenced by stage of disease, Hb, albumin, LDH,  $\beta_2$ -microglobulin values, splenectomy or molecular status at diagnosis.

The achievement of CR was associated with a trend for a higher freedom from progression (75.6% at 4 years versus 51.6%) ( $P = 0.11$ ).

On the contrary, the administration of rituximab, independently from its schedule, did significantly condition the 48-month TTP (83.4% cases treated with the anti-CD20 antibody free from progression versus 52.4% in the subgroup receiving cladribine alone;  $P = 0.04$ ) (Figure 1).

Molecular analysis at diagnosis was carried out on 42 cases; in 38 of them (90.4%), a molecular marker was available. Among patients who achieved a CR after cladribine and who were PCR positive at diagnosis, 33% became PCR negative after the purine analogue. On the other hand, in the subgroup of patients who were PCR positive at diagnosis and who achieved CR after rituximab, the PCR-negativity rate amounted to 81%. Nevertheless, the number of events in this group is too low to estimate if the eradication of minimal residual disease (MRD) would really play a relevant role in the outcome of these responding patients.



**Figure 1.** Kaplan–Meier analysis. Time to progression (TTP) of the entire series analyzed according to the administration of treatment with or without rituximab.

All patients were assessable for toxicity. The cladribine–rituximab association was relatively well tolerated. No differences in toxicity rate were observed according to rituximab administration or its schedule. Two cases experienced chills and fever during rituximab administration, which rapidly resolved after stopping infusion. One patient developed secondary malignancy (gastric carcinoma), with consequent death after 65 months. Hematological toxicity was mild (Table 2). Grade III neutropenia was recorded in three patients (6%); all these cases presented massive splenomegaly (longitudinal diameter > 20 cm). No infections were recorded. No secondary myelodysplastic syndromes were observed.

## discussion

Management of SMZL patients has not yet been standardized. Disease is often indolent, and frequently, a watch and wait policy has up until today been considered the best approach.

A specific therapy is required in the presence of symptoms. In the past, splenectomy was considered the treatment of choice, but more recent data has reported a change in the bone marrow infiltration and an increase in tumor burden after splenectomy [7]. Purine analogues (both fludarabine and pentostatin) constitute valid options, and significant response rates have been reported for cladribine in SMZL patients [12, 14, 24].

The advent of immunotherapy, in particular of anti-CD20 mAb, has produced a significant improvement in the outcome of several lymphoproliferative disorders. Inevitably, rituximab has been employed also in SMZL, demonstrating an effective antitumor activity [19]. Tsimberidou et al. demonstrated rituximab superiority when compared with chemotherapy alone: rituximab resulted in longer survival and failure-free survival (FFS) compared to chemotherapy. The ORRs were 88% with rituximab, 83% with rituximab plus chemotherapy and 55% with chemotherapy alone; the 3-year survival rates were 95%, 100% and 55%, respectively. The 3-year FFS rates were 86%, 100% and 45% in the rituximab, rituximab plus chemotherapy and chemotherapy-alone groups, respectively [16].

Furthermore, rituximab could be a possible treatment in HCV-related SMZL unresponsive to antiviral therapies. Given the central role of mature B cells in the pathogenesis of HCV-related NHLs, anti-CD20 seems an attractive therapeutic option, also considering its good toxicity profile.

In HCV-related NHLs, we reported a surprisingly high activity of rituximab in monotherapy, with 100% of clinical response and 50% of complete response, without any significant hepatic and extrahepatic toxicity [25].

MRD is considered a real prognostic index in predicting hematological relapse in lymphomas. On this basis, eradication

**Table 2.** Hematological toxicity associated with treatment

	Grade I–II	Grade III
Neutropenia	5	3
Anemia	7	1
Thrombocytopenia	2	1

of MRD could be considered an end point for improving quality of response and consequently the outcome even in SMZL.

Recently, we evidenced the relevant role of rituximab in eradicating MRD in HCL patients pretreated with cladribine, without significant toxicity [26]. According to these data, rituximab in combination with purine analogues could be an attractive therapeutic choice for SMZL.

In this light, we decided to review our series of 50 patients affected by SMZL who were treated with cladribine alone or plus anti-CD20, administered concomitantly with chemotherapy or after induction in our center. In this retrospective analysis, the comparison with or without rituximab was not planned before, and accordingly, the number of patients was not calculated for this question. Furthermore, different R-schedules were used. For these reasons, a statistical analysis is not really possible, it can only be a description, as is also true for CR rate and progression-free survival.

The results described above reported evidence that the schedule of anti-CD20 administration (concomitant or after cladribine) does not have any significant impact on quality of response. It is probably due to the long action activity of cladribine.

Moreover, these data indicate some relevant considerations that could further consolidate the role of rituximab in this histotype of lymphoma: (i) the addition of the anti-CD20 antibody to cladribine is able to increase the already good CR rate and prolong time without progression, independently of the schedule of administration and (ii) rituximab could act as an effective purging *in vivo* tool (almost when used after cladribine).

Notwithstanding our data indicating that the schedule of rituximab administration is not relevant to the outcome, we could hypothesize that the employment of the anti-CD20 antibody as consolidation after cladribine would be the most reasonable approach: our group previously reported that rituximab is effective in inducing good responses even when administered after a long time from cladribine induction [26]. Thus, it would be possible to differ the treatment with rituximab after evaluation of response to cladribine, with two main advantages (i) of avoiding expensive treatment and (ii) of reducing some possible infective complications in the immunosuppressive treatment, such as the multifocal encephalopathy that our group observed also in patients treated by rituximab [27].

Obviously, prospective and/or randomized trials appear fundamental in confirming these intriguing results.

## references

- Harris NL, Jaffe ES, Stein H et al. A revised European–American classification of lymphoid neoplasms. A proposal from the International Lymphoma Study Group. *Blood* 1994; 84: 1361–1392.
- Jaffe ES, Harris NL, Stein H, Vardiman JW. World Health Organization Classification of Tumours: Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues. Lyon, France: IARC 2001.
- Troussard X, Valensi F, Duchayne E et al. Splenic lymphoma with villous lymphocytes: clinical presentation, biology and prognostic factors in a series of 100 patients. *Groupe Francais d'Hematologie Cellulaire (GFHC). Br J Haematol* 1996; 93: 731–736.
- Nathwani B, Anderson J, Armitage J et al. Marginal zone B-cell lymphoma: a clinical comparison of nodal and mucosa-associated lymphoid tissue types. Non-Hodgkin's Lymphoma Classification Project. *J Clin Oncol* 1999; 17: 2486–2492.
- Berger F, Felman P, Thieblemont C et al. Non-MALT marginal zone B-cell lymphomas: a description of clinical presentation and outcome in 124 patients. *Blood* 2000; 95: 1950–1956.
- Thieblemont C, Felman P, Callet-Bauchu E et al. Splenic marginal-zone lymphoma: a distinct clinical and pathological entity. *Lancet Oncol* 2003; 4: 95–103.
- Franco V, Florena AM, Stella M et al. Splenectomy influences bone marrow infiltration in patients with splenic marginal zone lymphoma with or without villous lymphocytes. *Cancer* 2001; 91: 294–301.
- Andouin J, Le Tourneau A, Molina T et al. Patterns of bone marrow involvement in 58 patients presenting primary splenic marginal zone lymphoma with or without circulating villous lymphocytes. *Br J Haematol* 2003; 122: 404–412.
- Chacon J, Molloje M, Munoz E et al. Splenic marginal zone lymphoma: clinical characteristics and prognostic factors in a series of 60 patients. *Blood* 2002; 100: 1648–1654.
- Arcaini L, Lazzarino M, Colombo N et al. Splenic marginal zone lymphoma: a prognostic model for clinical use. *Blood* 2006; 107: 4643–4649.
- Lefrere F, Hermine O, Belanger C et al. Fludarabine: an effective treatment in patients with splenic lymphoma with villous lymphocytes. *Leukemia* 2000; 14: 573–575.
- Iannitto E, Minardi V, Calvaruso G et al. Deoxycoryformycin (pentostatin) in the treatment of splenic marginal zone lymphoma (SMZL) with or without villous lymphocytes. *Eur J Haematol* 2005; 75: 130–135.
- Virchis A, Mehta A. Splenic lymphoma with villous lymphocytes (SLVL) responding to 2-chlorodeoxyadenosine (2-CDA). *Br J Haematol* 1998; 100: 609.
- Lefrere F, Hermine O, Francois S et al. Lack of efficacy of 2-chlorodeoxyadenosine in the treatment of splenic lymphoma with villous lymphocytes. *Leuk Lymphoma* 2000; 40: 113–117.
- Riccioni R, Caracciolo F, Galimberti S et al. Low dose 2-CdA schedule activity in splenic marginal zone lymphomas. *Hematol Oncol* 2003; 21: 163–168.
- Hermine O, Lefrere F, Bronowicki JP et al. Regression of splenic lymphoma with villous lymphocytes after treatment of hepatitis C virus infection. *N Engl J Med* 2002; 347: 89–94.
- Keladi C, Rollor F, Park S et al. Response to antiviral treatment in hepatitis C virus associated marginal zone lymphomas. *Leukemia* 2004; 18: 1711–1716.
- Arcaini L, Orlandi E, Scotti M et al. Combination of rituximab, cyclophosphamide and vincristine induces complete hematologic remission of splenic marginal zone lymphoma. *Clin Lymphoma* 2004; 4: 250–252.
- Bennett M, Sharma K, Yegena S et al. Rituximab monotherapy for splenic marginal zone lymphoma. *Haematologica* 2005; 90: 856–858.
- Tsimberidou AM, Catovsky D, Schlette E et al. Outcomes in patients with splenic marginal zone lymphoma and marginal zone lymphoma treated with rituximab with or without chemotherapy or chemotherapy alone. *Cancer* 2006; 107: 125–135.
- Fabbri A, Gozetti A, Lazzi S et al. Activity of rituximab in refractory splenic marginal zone lymphoma complicated with autoimmune hemolytic anemia. *Clin Lymphoma Myeloma* 2006; 6: 496–499.
- Galimberti S, Brizzi S, Marnelli F et al. An advantageous method to evaluate IgH rearrangement and its role in minimal residual disease detection. *Leuk Res* 1999; 23: 921–929.
- Lauria F, Bocchia M, Marotta G et al. Weekly administration of 2-chlorodeoxyadenosine in patients with hairy-cell leukemia is effective and reduces infectious complications. *Haematologica* 1999; 84(1): 22–25.
- Bolam S, Orchard J, Oscier D. Fludarabine is effective in the treatment of splenic lymphoma with villous lymphocytes. *Br J Haematol* 1997; 99: 158–161.
- Cervetti G, Mechelli S, Riccioni R et al. High efficacy of Rituximab in indolent HCV-related lymphoproliferative disorders associated with systemic autoimmune diseases. *Clin Exp Rheumatol* 2005; 23(6): 877–880.
- Cervetti G, Galimberti S, Andreazzoli F et al. Rituximab as treatment for minimal residual disease in hairy cell leukaemia: extended follow-up. *Br J Haematol* 2008; 143: 296–298.
- Pelosini M, Focosi D, Fazzi R et al. Progressive multifocal leukoencephalopathy: report of three cases in HIV-negative haematological patients and review of literature. *Ann Hematol* 2008; 87: 405–412.