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2-Chlorodeoxyadenosine (2-CDA) therapy in previously untreated patients with follicular stage III–IV non-Hodgkin's lymphoma

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**See Appendix on page 798 for list of participating centers*

Summary

Purpose: This phase II multi-institutional trial was designed to assess response and toxicity of 2-chlorodeoxyadenosine (2-CDA) in patients with previously untreated follicular lymphoma. The clinical significance of detecting cells carrying the t(14;18) translocation (bcl-2/JH rearrangement) in peripheral blood and bone marrow by polymerase chain reaction (PCR) before, during and after treatment was also examined.

Patients and methods: Between May 1993 and October 1995, 37 patients were accrued: male/female: 15/22, median age 51 years (range: 20–78), stage III/IV: 9/28. Patients received a total 2-CDA dose of 0.7 mg/kg as continuous s.c. or i.v. infusions over 7 days, every 28 days for a maximum of 5 cycles. A total of 165 cycles were administered. In 25 patients, blood and bone marrow before, during and after treatment were available for PCR analysis of the bcl-2/JH rearrangements.

Results: All 37 patients were evaluable for response and toxicity. The overall response rate was 84% (95% confidence interval, 68%–94%) with 14% CR ($n = 5$) and 70% PR ($n = 26$) and a median time to treatment failure of 15.7 months. bcl-2/JH rearrangement in peripheral blood and/or bone marrow was found in 10/25 of patients (40%) before treatment and 5 of these became repeatedly negative after 2-CDA therapy. There was no apparent association between bcl-2/JH result and response. In 11 patients, 2-CDA was stopped because of progressive disease ($n = 4$), myelotoxicity (grade 2–3, $n = 4$), and other causes ($n = 3$, pulmonary embolism, metabolic disorder, and patient's decision). Four patients (11%) suffered from infections (grade 2–3). In 6 patients, persistent thrombocytopenia of 7.5 months (range: 3–21) occurred after completion of the 5 cycles.

Conclusion: 2-CDA is active in untreated follicular lymphomas, but time to treatment failure suggests no advantage compared with standard treatment and toxicity on haematopoietic stem cells appears to be more pronounced. Molecular remission is induced in a considerable proportion of patients with disappearance of the bcl-2/JH rearrangement, and its possible significance as a predictive factor for quality of response and relapse warrants further study.

Key words: bcl-2 rearrangement, 2-chlorodeoxyadenosine, cladribine, follicular lymphoma, t(14;18) chromosomal translocation

Introduction

Follicular lymphomas represent the most common subtype of malignant lymphomas. The cornerstone for diagnosis is, amongst others, the presence of a follicular growth pattern and, at the molecular level, the identification of a chromosomal translocation t(14;18) (q32;q21) that leads to the juxtaposition of the *bcl-2* oncogene on chromosome 18q21 adjacent to the immunoglobulin heavy-chain locus on chromosome 14q32. The clinical course of follicular lymphomas is comparable with other low grade lymphomas; the median survival ranges from 5–10 years and remissions can be induced either by single drug treatment or combination chemotherapy. In the majority of patients with advanced disease, however, none of the current treatment modalities result in prolonged relapse free survival and most patients ultimately die of disease [1–5]. New treatment strategies must therefore be developed. Intensive chemotherapy followed by autologous bone marrow transplantation is an interesting approach, which may prolong relapse free survival. However, efficacy in terms of cure has not yet been shown [6]. Assessment of residual disease at the molecular level (t(14;18) chromosomal translocation) is currently in experimental phase and might be of value in the prediction of relapses and, perhaps, it will help to improve the timing of treatment [7–10].

In the last decade, various new drugs, mainly purine analogues, have shown activity in a great proportion of patients with low grade lymphomas. 2-Chlorodeoxyadenosine (2-CDA) is one of these purine analogues [11–13], which is highly active in pretreated low-intermediate grade non-Hodgkin's lymphoma (NHL) [14–

17]. In two studies follicular lymphoma appeared to be more responsive to 2-CDA with 50% responses compared to 30% in patients with diffuse growth pattern [15, 17].

In 1993, the Swiss Group for Clinical Cancer Research (SAKK) initiated this phase II trial of 2-CDA in non-pretreated patients with advanced follicular NHL to study activity, toxicity, remission duration and survival. As a secondary objective, the prognostic value of residual disease in bone marrow and peripheral blood by the PCR examination of mononuclear cells carrying the (14;18) translocation (bcl-2/JH rearrangement) was assessed.

Patients and methods

Study design

This study was a multicentre, phase II trial initiated by the SAKK in May 1993. Ten centres participated in this trial (see Appendix). The protocol was approved by the local ethics committees of each participating centre. The study was performed in keeping with good clinical practice and informed consent was given by all patients. The trial was closed for accrual in October 1995.

Eligibility criteria

Patients were required to have newly diagnosed stage III–IV follicular small, mixed or large cell NHL (IWF B-D [International Working Formulation for Clinical Usage] or follicle centre cell lymphoma, follicular grade I–III according to the REAL classification [revised European and American Lymphoma Classification]). Histological diagnosis was performed immediately before enrollment. All diagnoses were confirmed by central pathology review (K.B.). Age limits were originally set between 18 and 80 years, but due to 2-CDA induced toxicity (pneumonia, grade 3, and fatal pulmonary embolism) the upper age limit was reduced to 72 years after enrollment of 18 patients. Patients were not included if they had a life expectancy of less than 3 months, if they had had prior malignancy other than non-melanomatous skin cancer or adequately treated stage I *in situ* cervical cancer, if they had another life threatening disease (kidney dysfunction, cardiac failure etc.) or if they had peripheral blood cytopenia (leucocyte count $<3.0 \times 10^9$/l, neutrophil count $<1.0 \times 10^9$/l and thrombocyte count $<100 \times 10^9$/l) unless this was clearly related to bone marrow infiltration with NHL.

Pretreatment investigations

The extent of disease was determined by standardised staging evaluation, which included CT scanning of the chest, abdomen and pelvis, and bone marrow aspiration and trephine biopsy. In the case of gastrointestinal symptomatology or involvement of Waldeyer's ring, a gastroscopy and head and neck evaluation was performed. Kidney function (creatinine and urine analysis), liver enzymes, and LDH were measured and lymphocyte immunophenotyping (CD4, CD8), protein electrophoresis and electrocardiogram performed before 2-CDA therapy was initiated.

Drug therapy

Patients were treated with 2-chlorodeoxyadenosine (cladribine) (Lipomed, Basel, Switzerland) at a dose of 0.1 mg/kg/day diluted in 0.9% saline solution as continuous i.v. or s.c. infusions over 7 days. For subcutaneous administration, 2-CDA solution was injected using a Disetronic Infusor® pump system, kindly provided by Disetronic AG (Burgdorf, Switzerland). 2-CDA cycles were repeated at 4 week intervals for a maximum of 5 cycles. Treatment was delayed if the neutrophil count was less than 1.0×10^9/l and/or the platelet count less than 100×10^9/l (until recovery), unless the cause was clearly due to infiltration of the bone marrow by the NHL. No antimicrobial prophylaxis was given, and no concomitant therapy such as antiemetics or steroids were administered. Treatment was discontinued in case of disease progression after 3 cycles.

Response criteria

All initial sites of disease were reassessed after 3 and 5 treatment cycles. A complete remission (CR) was defined as absence of detectable disease on two measurements separated by an interval of at least 4 weeks. In particular, all lymph nodes had to reveal normal size as judged by physical examination and CT scanning of the chest, abdomen, and pelvis, and the bone marrow biopsy had to show less than 20% lymphocytes and no evidence of abnormal lymphoid infiltration. A partial remission (PR) was defined as a reduction in all involved areas of disease by at least 50%. Any response less than that sufficient to qualify as a PR was no change (NC). Progressive disease was defined as a $\geq 25\%$ increase in measurable disease or the occurrence of new lesions.

Toxicity criteria

Toxicity was evaluated according to World Health Organization (WHO) criteria [18]. Physical examination and full blood count including differential were done weekly. Serial immunophenotyping of peripheral blood lymphocytes was performed before each treatment cycle and at regular intervals after completion of therapy.

Statistical analyses

All patients were evaluated for toxicity, response and survival. Time to treatment failure was calculated from date of enrollment to either disease progression/relapse, discontinuation of treatment for toxicity or death from any causes. Event-free survival was calculated from the date of enrollment to disease progression, relapse, or death and overall survival from the date of enrollment to death for any cause. Response duration was calculated from the date of response until relapse, progression or death. The cutoff for all the above calculations was March 15, 1996. Survival curves were compared using the log-rank test. Contingency tables were analysed by Fisher's exact test, while continuous variables were analysed by Wilcoxon signed rank test (one sample) or Wilcoxon rank sums test (two samples). The level of significance was $P = 0.05$.

Assessment of the bcl-2/JH rearrangement

Whole blood (5 ml) and bone marrow (2 ml) samples, stored at −20 °C, were treated with lysis buffer (0.32 M sucrose, 10 mM Tris-HCl pH 7.5, 5 mM $MgCl_2$, 1% Triton X-100) to remove red cells. The cell pellet obtained by centrifugation (15 min at 3000 rpm) was then resuspended in 100 mM NaCl, 25 mM EDTA, 0.5% SDS and 200 µg/ml proteinase K (Sigma, St. Louis, USA) and incubated overnight at 37 °C. DNA was extracted by phenol-chloroform and precipitated in ethanol. Small pellets were resuspended in a digestion buffer (1 × PCR buffer, 0.5% Tween 20 and 200 µg/ml proteinase K for 100 µl) and incubated at 56 °C for 90 min and then at 94 °C for 20 min to inactivate enzymatic activity. DNA presence was tested with a PCR assay using PCO4 (5'CAACTTCATCCACGTTCACC-3') and GH20 (5'-GAAGAGCCAAGGACAGGTAC-3') primers (Perkin Elmer, Norwalk, USA) which amplifies a 268 bp segment of the β globin gene. 1.5 µg of phenol-chloroform extracted DNA or 5 µl of digestion buffer extracted DNA were analysed.

Nested PCR reactions were performed in 50 µl volumes including 1 × PCR buffer (50 mM KCl, 10 mM Tris-HCl pH 8.4, 2.5 mM $MgCl_2$), 200 µM of each deoxynucleotide (Pharmacia, Uppsala, Sweden), 25 pmol of each primer and 1.25 U Taq polymerase (GIBCO, Gaithesburg, USA). The first round of the nested PCR amplification was performed for 30 cycles with the outer primers 5'ACCTGAGGAGACGGTGACC-3' for the JH region and 5'CAGCCTTGAAACATTGATGG-3' for MBR (Pharmacia Biotech, Rosendaal, the Netherlands). Each cycle included 45 sec of denaturation at 95 °C, 1 min of annealing at 60 °C and 2 min of extension at 72 °C. A touch-down PCR technique was used starting from annealing temperature of 60 °C then decreased by 1 °C every two of the first eight cycles. Of first round DNA product, 5 µl was re-amplified for 30 cycles with inner primers 5'CAGGGTTCCTTGGCCCCAG-3' for the JH region and 5'-AGTTGCTTTACGTGGCCTGT-3' for MBR (Pharmacia Biotech). Each cycle included 45 sec of denaturation at 94 °C, 1 min of annealing at 57 °C and 1 min of extension at 72 °C. DNA amplification always started with 10 min of initial denaturation at 95 °C and finished with 10 min of final extension at 72 °C. Patient samples were always analysed together with human B-cell lymphoma cell line DOHH2 (kindly provided by Dr. F. E. Cotter, London, UK) as a positive control and the reaction mixture with no DNA as negative reagent control. Of the nested PCR product, 10 µl were loaded in 2% agarose electrophoresis gel containing ethidium bromide and visualized under ultraviolet light.

Results

Study population

Thirty-seven patients with previously untreated follicular NHL stage III–IV were enrolled between May 1993 and October 1995. In 36 patients the diagnosis was established by lymph node biopsy and in one patient by bone marrow biopsy alone. In all cases results of pathology review was in accordance with the diagnosis made by the local pathologist. There were 16 patients with follicular small (IWF B, REAL, follicle centre cell, follicular grade I), 18 patients with follicular mixed (IWF C, REAL, follicular grade II) and 3 patients with follicular large cell lymphomas (IWF D, REAL, follicular grade III). One hundred and sixty-five cycles of 2-CDA were administered to 37 patients. The median number of cycles was 5 (range, 2 to 5 cycles), with 26 patients receiving the full 5 cycles of 2-CDA according to protocol. Treatment was stopped prematurely in 11 patients due to death [progressive disease ($n = 2$), pulmonary embolism ($n = 1$) and metabolic disorder (diabetes mellitus, $n = 1$)], progressive disease ($n = 2$), toxicity [persistent thrombocytopenia ($n = 3$) or neutropenia ($n = 1$)] and patient's decision ($n = 1$). The median actual dose-intensity for all patients was 0.17 mg/kg/week (range 013–0.21 mg/kg/week) and did not differ between responders and non-responders. Patient characteristics are listed in Table 1.

Response to therapy

The overall response rate in 37 patients was 84% (95% confidence interval (CI), 68% to 94%), with a CR rate of 14% (95% CI, 5%–29%) and a PR rate of 70% (95% CI, 53%–84%). All 31 responders had reached at least a PR after three cycles of 2-CDA. Table 2 summarises patient response by histological subtype. Seven of 8 complete remissions occurred in follicular mixed lymphomas (IWF C, REAL, follicle centre cell, follicular grade II) ($P = 0.02$). On the cutoff date 71% of the responders were still under remission. The 75% quantile response duration was 15.2 months.

After 5 cycles of 2-CDA, the patients were observed without receiving further therapy until relapse or progression. The median time to treatment failure was 15.7 months (Figure 1). The median follow-up time (from enrollment) of all patients was 20.3 months. The one year event-free and overall survival rates were 73% (27/37) and 89% (33/37), respectively.

We tested several parameters known to be prognostic factors associated with event-free survival [1, 19]. Although the median event-free survival has not been

Table 1. Patient characteristics ($n = 37$).

Characteristic	No.
Age, years	
Median	51
Range	20–78
Gender	
Male	15
Female	22
Histology	
IWF B, REAL, follicle centre cell, follicular grade I	16
IWF C, REAL, follicle centre cell, follicular grade II	18
IWF D, REAL, follicle centre cell, follicular grade III	3
Ann Arbor stage	
III	9
IV	28
Number of nodular sites	
1–2	5
3–4	15
⩾5	17
Bone marrow involvement	
Present	26
Absent	11
Number of patients with	
Splenomegaly	19
Hepatomegaly	8
Criteria for high tumor burden	
Tumor mass >7 cm	14
B-symptoms	12
Liver function tests	
ASAT (⩽1.5 × upper normal limit)	35
ASAT (>1.5 × upper normal limit)	2
Serum lactate dehydrogenase (LDH) level	
Normal – ⩽1.5 × upper normal limit	32
>1.5 × upper normal limit	5

Table 2. Response rate by histological subtype.

IWF	Description: follicular	REAL*	N patients	Response			Response duration (months) median (range)
				Overall	CR	PR	
B	Small cell	I	16	14	1	13	13.9+ (3.7–21.3)
C	Mixed cell	II	18	15	7	8	16.7+ (1.1–33.4)
D	Large cell	III	3	2	0	2	19.5+ (17.5–21.9)

* REAL, follicle centre cell, follicular grade I–III.

reached, it was reduced in male patients ($P = 0.05$), in patients with worse performance status (PS 0 *versus* PS 1–2, $P = 0.003$), in patients with spleno- and/or hepatomegaly ($P = 0.03$), and if LDH was increased at study entry ($<1.5 \times$ UNL *versus* $\geq 1.5 \times$ UNL, $P = 0.0002$). Age, presence of B-symptoms, Ann Arbor stage (III *versus* IV), and bone marrow involvement had no prognostic value in our series, probably due to the low patient number and relatively short observation time.

The 2-CDA activity on lymphocytes as well as CD4 and CD8 positive cells was assessed regularly during and after 2-CDA therapy. The median lymphocyte count including CD4 and CD8 cells decreased throughout the period of treatment attaining the lowest value (total lymphocyte count: 0.4×10^9/l; CD4: 0.11×10^9/l; CD8: 0.08×10^9/l) at its completion. The total lymphocyte count did not recover to pretreatment values until 12 months after the completion of the 2-CDA therapy ($P < 0.012$) (Figure 2).

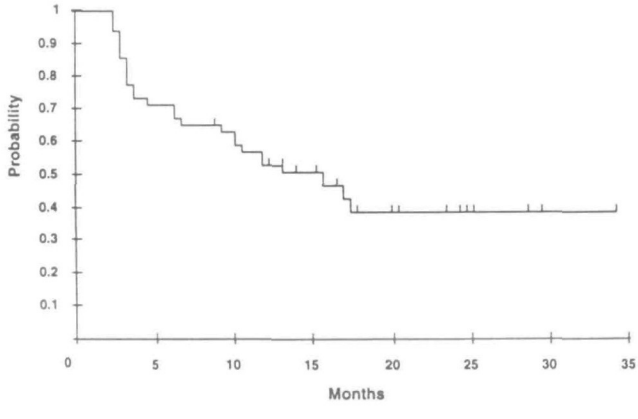

Figure 1. Time to treatment failure ($n = 37$).

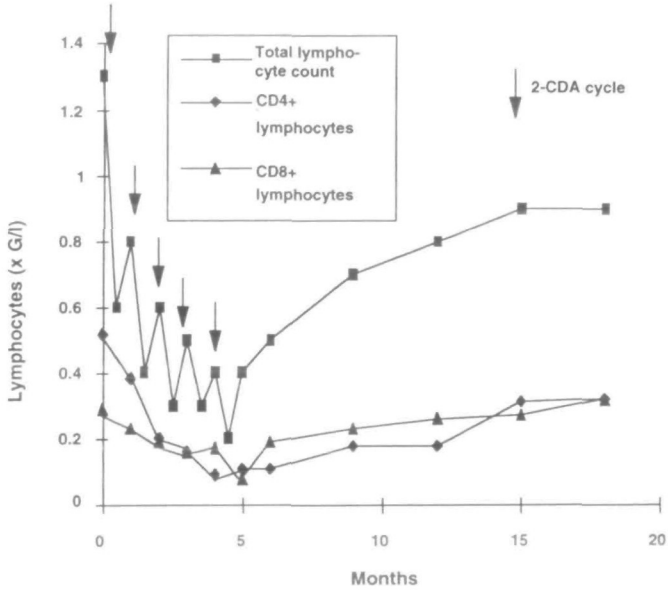

Figure 2. Median lymphocyte counts (total, CD4 and CD8 positive cells).

Bcl-2/JH rearrangement

One of the study objectives was to define whether the presence of mononuclear cells carrying the t(14;18) translocation in PB and BM would have any clinical significance in predicting relapse. Seventy-seven PB and 70 BM samples from 25 patients were available for investigation. Bcl-2/JH rearrangement was found in 10/25 of patients (40%) at study entry and 5 became negative after 2-CDA therapy. No progression has been seen in these 5 patients. Conversely, one of 3 patients remaining positive for bcl-2 rearrangement relapsed after 8 months. The results are detailed in Table 3. Besides, among the 25 patients, there were only 3 non-responders, whose bcl-2/JH results were negative.

Toxicity

Non-hematologic toxicity was mild. In particular, nausea or vomiting did not occur and no patient had mucositis, neurologic symptoms, or alopecia. No drug related renal or hepatic toxicity was encountered. In 29 patients (126 cycles), 2-CDA was administered subcutaneously to out-patients using a Disetronic® pump system. This system was well tolerated except for moderate exanthema and induration at the injection site in 69 of 126 cycles.

Grade 3 or 4 neutropenia was experienced by 8 of 37 patients, in 12 of 35 cycles. Recovery to baseline values was usually seen by day 28. In one patient therapy had to be delayed by 4 days because of a low neutrophil count. Grade 3 and 4 thrombocytopenia was seen in 4 of 37 patients (4 of 15 cycles). No haemor-

Table 3. Bcl-2/JH rearrangement in mononuclear cell fraction of peripheral blood (PB) and bone marrow (BM) assessed by PCR amplification (× = no DNA available for bcl-2/JH rearrangement analysis).

UPN	PB/ BM	Before therapy	After 3 cycles	After 5 cycles	At relapse	Histological BM involvement	Remission duration (months)
1	PB	+	–	–		+	33.4+
	BM	+	–	–			
7	PB	+	–	–		+	25.8+
	BM	–	–	–			
19	PB	+	×		×	+	1.6
	BM	×	×		–		
20	PB	+	–	–		+	18.2+
	BM	+	–	×			
21	PB	+	+	+		+	17.4+
	BM	+	+	+			
28	PB	+	–	–		+	13.1+
	BM	+	–	×			
32	PB	+	×	–		–	10.3+
	BM	–	×	–			
35	PB	–	–			–	11.8+
	BM	+	×				
36	PB	+	+	+		+	9.1+
	BM	×	+	+			
39	PB	+	×	×	+	+	8.3
	BM	×	×	+	+		

rhages occurred, and no platelet support was necessary. Median values of neutrophils and platelets during and after completion 2-CDA therapy are shown in Figure 3.

Although there was no evidence of cumulative myelosuppression on day 14 for neutrophils (neutropenia of WHO grade ≥3 in 4/37 patients after cycle 1, in 2/35 patients after cycle 3 and in 0/26 patients after cycle 5), persistent thrombocytopenias (median 73 × 10^9/l, range 15–93) occurred in 9 patients. In six patients, platelet counts still decreased one to six months after completion of the 2-CDA therapy. In a further 3 patients 2-CDA had to be discontinued after the 3rd ($n = 1$), or the 4th ($n = 2$) cycle of 2-CDA. This myelotoxicity showed no recovery after 7.5 months (median, range 3–21 months). Age, gender, stage, performance status and presence of B-symptoms, LDH and ASAT levels, splenomegaly, hepatomegaly, lymphocyte and neutrophil counts before and during treatment, response, histological subtype, number of nodular sites, bone marrow involvement as well as tumor burden were not associated with a higher risk to acquire delayed persistent thrombocytopenia. In one patient, thrombocytopenia was associated with neutropenia which lasted for 21 months. Further investigations including cytogenetic analysis and stem cell cultures led to the diagnosis of a myelodysplastic syndrome with monosomy of chromosome 7 and trisomy of chromosome 8.

Infectious complications were rare. Nine patients (11 cycles) experienced grade 1–2 infections including bacterial urinary infection ($n = 3$), herpes genitalis ($n = 2$), herpes zoster ($n = 1$), viral pharyngitis ($n = 4$), and paronychia of the finger ($n = 1$). One patient suffered from pneumonia (grade 3). No patient developed a significant infection between cessation of 2-CDA treatment after achieving a response until cut off (March 15, 1996) or resumption of chemotherapy because of relapse.

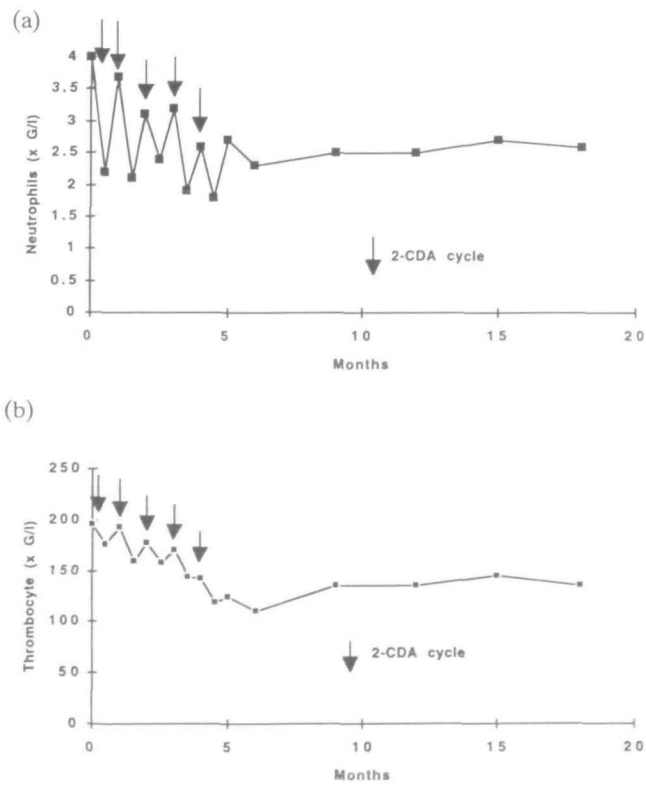

Figure 3. Median neutrophil (a) and thrombocyte (b) counts are shown during and after therapy.

Discussion

The activity of 2-CDA in low grade lymphoma has been studied by several investigators. Overall remission rates are in the range of 22%–70% in pretreated and 60%–100% in previously untreated patients with low grade lymphomas [12, 14–17, 20–26]. Almost all studies assembled very heterogeneous groups of patients with different lymphoma types. Additionally, there is so far only little information regarding time to treatment failure and event-free survival, and experience with this drug in non-pretreated NHL patients is still limited.

In the present study we examined a homogenous group of previously untreated patients with advanced follicular lymphoma. All patients required therapy due to high tumour burden and/or bone marrow involvement. The results show 2-CDA to have significant activity as a single agent with an overall response rate of 84% (14% CR). Complete remissions appeared to occur more frequently in patients with mixed cell follicular lymphomas (IWF C, REAL, follicle centre cell, follicular grade II) ($P = 0.02$). Our response rate is in agreement with the recent study reported by Saven et al. [27], in which 10 patients with previously untreated follicular NHL are described (3 patients with CR and 5 with PR). In our study, all responders had reached a PR after three cycles of 2-CDA, and it appears therefore that continuation of the drug in patients with no response after 3 cycles is not indicated. In advanced follicular lymphomas, time to treatment failure, event-free and overall survival are the most important end points. The median time to treatment failure for our 37 assessable patients in the present study was 15.7 months which is very similar to that obtained after other therapies. Median event-free and overall survival have not yet been reached.

Very recently, a trial assessing another purine analogue, fludarabine phosphate, has been reported in previously untreated patients with follicular NHL [28]. The overall response of 65% (however, with a greater rate of CRs of 37%) and the median time to treatment failure of 9 months were similar to our results. When comparing these data to previously reported studies with cyclophosphamide or vinca alkaloids, 2-CDA and fludarabine phosphate appear, however, to possess similar activity [5].

The t(14;18)(q32;q21) chromosomal translocation is a consistent feature of follicular lymphomas and pre-

sents an opportunity for the molecular monitoring of subclinical disease. This translocation juxtaposes the *bcl-2* proto-oncogene on chromosome 18q21 with the joining region (JH) of the immunoglobulin heavy chain on chromosome 14q32 and results in overexpression of a functionally normal bcl-2 protein, which in turn leads to a reduction of apoptosis and a growth advantage for the neoplastic clone. The majority of breakpoints on chromosome 18 are clustered in two areas; the major breakpoint region (MBR) in approximately 60% and the minor cluster region (mcr) in 10%–20% of cases [29]. The PCR using consensus JH primer and primers specific to one or more *bcl-2* gene breakpoints regions, is a sensitive and reproducible assay for detecting the bcl-2/JH rearrangement [30]. This rearrangement was detected in the BM and/or PB mononuclear cells from 10 of 25 patients (40%). This relatively low frequency may be explained by the fact that PCR analyses generally underestimate the rearrangement frequency [31]. In a survey of follicular lymphoma, in which a translocation frequency of 76% was determined by cytogenetics, PCR analyses led to a detection of only 46% when examining the major breakpoint region [31]. This is presumably due to breakpoints falling outside the range of primers used. Nevertheless, we were interested to know whether a conversion from positive to negative correlated with clinical response and long remission duration. In our study, 5 patients showed this pattern, but another 3 patients showed PCR positivity which remained unchanged during treatment. As shown in Table 3, there is so far no apparent association between this result and outcome. These data are in agreement with previous studies showing the absence of predictive value when the bcl-2/JH rearrangement was measured in blood or bone marrow mononuclear cells [32, 33]. Indeed, no study has yet shown that a clearance of bcl-2 rearranged cells from BM and PB results in a better survival. Further investigations are therefore needed to definitely evaluate a possible significance of (14;18) translocation assessments at regular intervals.

Non-hematological toxicity of 2-CDA was moderate. The infection rate was low and there were no other significant adverse effects. In particular, neurotoxicity frequently observed when using purine analogues was not seen and no alopecia or severe nausea were encountered. Infections have been reported to occur frequently after 2-CDA therapy in pretreated patients [15, 17, 34], but in this study, infections (grade 2–3) were seen in only 4 patients (11%).

The most significant toxicity was myelotoxicity. Median platelet, neutrophil and total lymphocyte counts had not recovered to baseline value 13 months after the cessation of 2-CDA. In particular, a persistent and delayed thrombocytopenia occurred in 9/37 (24%) patients, and in one case a myelodysplastic state was diagnosed. Severe treatment-related thrombocytopenia has also been reported by Carson et al. [35] using a larger dose of 2-CDA (0.1–1 mg/kg over 14 days) in pretreated patients and, in a recent study of non-pretreated chronic lymphocytic leukaemia patients reported by Saven et al. [36], grade 3–4 thrombocytopenia occurred in 23%–40% of cases. The mechanism by which this important adverse effect is induced is not clear and in our study, we found no factor associated with a greater risk of persistent thrombocytopenia.

In conclusion, these results show that 2-CDA is effective in previously untreated follicular lymphomas, although in terms of response rate and remission duration, the drug does not appear to be more active than standard therapy. The persistent thrombocytopenia, in some patients is of concern and further studies, perhaps with a reduced 2-CDA dose (0.5 mg/kg/cycle), are needed before a clear role for 2-CDA in the treatment of low grade lymphomas can be established.

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*Appendix

Participating centres: Hôpital Cantonal Universitaire, University of Geneva, P. Alberto; Policlinico Borgo Roma, Verona, Italy, G. Perona; Ospedale San Giovanni, Bellinzona, F. Cavalli; Christie Hospital, Manchester, UK, D. Crowther; Klinikum Nord, Institute of Medical Oncology, Nürnberg, Germany, C. Falge; Institute of Medical Oncology, Inselspital, University of Berne, M.F. Fey; Centre Hospitalier Universitaire Vaudois, University of Lausanne, S. Leyvraz; Dept. of Internal Medicine, Thun, J.M. Lüthi; Dept. of Internal Medicine, Bruderholz, V. Meier; Baden, Aargau, A. Streit.

References

1. Gallagher CJ, Lister TA. Follicular non-Hodgkin's lymphoma. Bailleres Clin Haematol 1987; 1: 141–55.
2. Spinolo JA, Cabanillas F, Dixon DO et al. Therapy of relapsed or refractory low-grade follicular lymphomas: Factors associated with complete remission, survival and time to treatment failure. Ann Oncol 1992; 3: 227–32.
3. Rohatiner AZS, Lister TA. The management of follicular lymphomas. Drugs 1994; 47 (Suppl 6): 10–8.
4. Johnson PWM, Rohatiner AZS, Whelan JS et al. Patterns of survival in patients with recurrent follicular lymphoma: A 20-year study from a single center. J Clin Oncol 1996; 13: 140–7.
5. Gisselbrecht C, Cosset JM. Treatment of low-grade non-Hodgkin's lymphomas. In Solal-Céligny P, Brousse N, Reyes F et al. (eds): Non-Hodgkin's Lymphomas. London: Manson 1993; 317–36.
6. Rohatiner AZS, Johnson PWM, Price CGA et al. Myeloablative therapy with autologous bone marrow transplantation as consolidation therapy for recurrent follicular lymphoma. J Clin Oncol 1994; 12: 1177–84.
7. Lambrechts AC, Hupkes PE, Dorssers LCJ et al. Clinical significance of t(14;18)-positive cells in the circulation of patients with stage III or IV follicular non-Hodgkin's lymphoma during first remission. J Clin Oncol 1994; 12: 1541–6.
8. Gribben JG, Neuberg D, Freedman AS et al. Detection by

polymerase chain reaction of residual cells with the bcl-2 translocation is associated with increased risk of relapse after autologous bone marrow transplantation for B-cell lymphoma. Blood 1993; 81: 3449–57.
9. Gribben JG, Neuberg D, Barber M et al. Detection of residual lymphoma cells by polymerase chain reaction in peripheral blood is significantly less predictive for relapse than detection in bone marrow. Blood 1994; 83: 3800–7.
10. Johnson PWM, Price CGA, Smith T et al. Detection of cells bearing the t(14;18) translocation following myeloablative treatment and autologous bone marrow transplantation for follicular lymphoma. J Clin Oncol 1994; 12: 798–805.
11. Cheson BD. New chemotherapeutic agents for the treatment of low-grade non-Hodgkin's lymphomas. Semin Oncol 1993; 20: 96–110.
12. Pott-Hoeck C, Hiddemann W. Purine analogs in the treatment of low-grade lymphomas and chronic lymphocytic leukemias. Ann Oncol 1995; 6: 421–33.
13. Saven A, Piro LD. 2-Chlorodeoxyadenosine: A newer purine analog active in the treatment of indolent lymphoid malignancies. Ann Int Med 1994; 120: 784–91.
14. Hickish T, Serafinowski P, Cunningham D et al. 2'-Chlorodeoxyadenosine: Evaluation of a novel predominantly lymphocyte selective agent in lymphoid malignancies. Br J Cancer 1993; 67: 139–43.
15. Kay AC, Saven A, Carrera CJ et al. 2-Chlorodeoxyadenosine treatment of low-grade lymphomas. J Clin Oncol 1992; 10: 371–7.
16. Hoffman M, Tallman MS, Hakimian D et al. 2-Chlorodeoxyadenosine is an active salvage therapy in advanced indolent non-Hodgkin's lymphoma. J Clin Oncol 1994; 12: 788–92.
17. Betticher DC, Fey MF, Von Rohr A et al. High incidence of infections after 2-chlorodeoxyadenosine (2-CDA) therapy in patients with malignant lymphomas and chronic and acute leukaemias. Ann Oncol 1994; 5: 57–64.
18. World Health Organization. Handbook for Reporting Results of Cancer Treatment. WHO offset publication no. 48. Geneva: WHO 1979.
19. Coiffier B, Bastion Y, Berger F et al. Prognostic factors in follicular lymphomas. Semin Oncol 1993; 20: 89–95.
20. Liliemark J, Juliusson G. Intermittent infusion of cladribine (CdA) in previously treated low grade non-Hodgkin's lymphoma (LG-NHL): Response, toxicity and survival. Proc Am Soc Clin Oncol 1995; 14: 191.
21. Khan A, Schiller G, Levine A et al. 2-Chloro-2'-deoxyadenosine (2-CDA), an active agent in the treatment of advanced relapsed or refractory low to intermediate grade non-Hodgkin's lymphoma. Proc Am Soc Clin Oncol 1994; 13: 386.
22. Rummel M, Leimer L, Hoffknecht M et al. 2-CDA treatment of untreated low grade non-Hodgkin lymphomas – preliminary results of a phase II study. Blood 1995; 86 (Suppl 1): 56a.
23. Kong L, Rademarker A, Hakimian D et al. Long-term follow-up after 2-chlorodeoxyadenosine therapy in previously treated advanced non-Hodgkin's lymphoma (NHL). Proc Natl Acad Sci USA 1996; 15: 425 (Abstr 1311).
24. Fridrik MA, Linkesch W, Jäger G et al. Response and toxicity of cladribine (2-CDA) as first line treatment of low-grade non-Hodgkin's lymphomas (NHL). Preliminary data of an Austrian multicentre trial. Blood 1995; 86 (Suppl 1): 56a.
25. Piro LD, Petroni G, Barcos M et al. Bolus infusion 2-chlorodeoxyadenosine (2-CDA) as first-line therapy of low grade non-Hodgkin's lymphoma (NHL): CALGB 9153. Blood 1995; 86 (Suppl 1): 274a.
26. Jäger G, Fridrik MA, Kienzer HJ et al. Response and toxicity of cladribine (2-CDA) as first line treatment of low grade non-Hodgkin's lymphomas (NHL). Preliminary data of an Austrian multicenter trial. Proc Natl Acad Sci USA 1996; 15: 410 (Abstr 1253).
27. Saven A, Emanuele S, Kosty M et al. 2-Chlorodeoxyadenosine activity in patients with untreated indolent non-Hodgkin's lymphoma. Blood 1995; 86: 1710–6.
28. Solal-Céligny P, Brice P, Brousse N et al. Phase II trial of fludarabine monophosphate as a first-line treatment in patients with advanced follicular lymphoma: A multicenter study by the groupe d'étude des lymphomes de l'adulte. J Clin Oncol 1996; 14: 514–9.
29. Cotter F, Price C, Zucca E et al. Direct sequence analysis of the 14q+ and 18q- chromosome junctions in follicular lymphoma. Blood 1990; 76: 131–5.
30. Yuan R, Dowling P, Zucca E et al. Detection of bcl-2/JH rearrangement in follicular and diffuse lymphoma: Concordant results of peripheral blood and bone marrow analysis at diagnosis. Br J Cancer 1993; 67: 922–5.
31. Turner GE, Ross FM, Krajewski AS. Detection of t(14;18) in British follicular lymphoma using cytogenetics, Southern blotting and the polymerase chain reaction. Br J Haematol 1995; 89: 223–5.
32. Price CGA, Meerabux J, Murtagh S et al. The significance of circulating cells carrying t(14;18) in long remission from follicular lymphoma. J Clin Oncol 1991; 9: 1527–32.
33. Gribben JG, Freedman AS, Woo SD et al. All advanced stage non-Hodgkin's lymphomas with a polymerase chain reaction amplifiable breakpoint of bcl-2 have residual cells containing the bcl-2 rearrangement at evaluation and after treatment. Blood 1991; 78: 3275–80.
34. Keating MJ. Immunosuppression with purine analogues – the flip side of the gold coin. Ann Oncol 1993; 4: 347–8.
35. Carson AD, Wasson DB, Beutler E. Antileukemic and immunosuppressive activity of 2-chloro-2-deoxyadenosine. Proc Natl Acad Sci 1984; 81: 2232–6.
36. Saven A, Lemon RH, Kosty M et al. 2-Chlorodeoxyadenosine activity in patients with untreated chronic lymphocytic leukemia. J Clin Oncol 1995; 13: 570–4.

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