

Recent Clinical Trials of Cladribine in Hematological Malignancies and Autoimmune Disorders

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Abstract: The purine nucleoside analog – cladribine (2-chlorodeoxyadenosine, 2-CdA) is a cytotoxic agent with high activity in lymphoid and myeloid malignancies. It is also an effective drug in some autoimmune disorders. 2-CdA is usually administered intravenously in continuous or 2-hour infusion. Recently however, new formulation of this agent has been developed for subcutaneous and oral administration. 2-CdA is widely established as first line standard treatment for hairy cell leukemia. Moreover several clinical trials have demonstrated that this agent, used alone or in combination with other cytotoxic drugs, showed good efficacy and acceptable toxicity profile in the treatment of chronic lymphocytic leukemia, Waldenström macroglobulinemia, low-grade non-Hodgkin's lymphoma and acute myeloid leukemia. Moreover, some studies indicate that 2-CdA has some activity in progressive multiple sclerosis and other autoimmune disorders including autoimmune hemolytic anemia, rheumatoid arthritis, systemic lupus erythematosus, psoriasis and in patients with refractory factor VIII inhibitors. This review article will summarize the results of recent clinical trials with 2-CdA in hematological malignancies, multiple sclerosis and other autoimmune diseases.

Keywords: Cladribine, clinical trials, leukemia, lymphoma, multiple sclerosis, autoimmune disease.

INTRODUCTION

Cladribine (2-chlorodeoxyadenosine, 2-CdA) is a purine nucleoside analog (PNA) resistant to deamination by adenosine deaminase (ADA) [1,2]. It was synthesized in 1980 by Carson *et al.* [3] and more recently by Kazimierczuk *et al.* [4] by a simple substitution of a chlorine atom with a hydrogen atom at the position 2 of the purine ring of deoxyadenosine. 2-CdA is a prodrug and its intracellular phosphorylation is necessary for cytostatic effect to occur. It is phosphorylated by deoxycytidine kinase (dCK) and accumulates as 2-chlorodeoxyadenosine triphosphate (2-CdATP) [5]. High activity of this enzyme in lymphocytes along with their low 5-nucleotidase (5'-NT) activity probably explains its relatively high selectivity for lymphoid cells. The nucleoside that is formed does not readily exit from the cells through the cell membrane and therefore accumulated inside the cell. This metabolite disrupts cell metabolism by incorporating into the DNA of the actively dividing cells and freezes cell cycles at S phase [6].

In contrast to other antineoplastic drugs, 2-CdA is cytotoxic to both proliferating and quiescent cells. In quiescent cells 2-CdA-TP interferes with proper repair of DNA and leads to a total disruption of cellular metabolism *via* accumulation of breaks in DNA strand, which in turn leads to p53 expression and consequently to induction of apoptosis [7,8]. Apoptosis induced by 2-CdA can be mediated either *via* DNA damage and p53 protein expression

or directly *via* mitochondrial permeability transition pore (mtPTS) [9]. Inhibition of DNA repair and accumulation of DNA breaks lead to p53 expression, which plays a key role in control of apoptosis and cell cycle and influences the bcl-2 protein family with antiapoptotic properties as well as bcl-2 like proteins such as bax, bcl-xs and bak, which have proapoptotic action [7,11].

The best method for 2-CdA administration remains to be determined. In the majority of studies in hairy cell leukemia (HCL) patients, the drug was given in 2-hour continuous intravenous infusion at a dose of 0.14 mg/kg per day for 7 days [12]. Further observations have suggested that similar results could be obtained after giving 2-CdA in 2-hour infusions for 5 or 7 days [13,14]. In a preliminary, nonrandomized study we have shown previously that there were no statistically significant differences in response rate and toxicity of 2-hour and 24-hour infusions of this drug in patients with the classic form of HCL [14]. Pharmacokinetics data also suggest that 2-CdA may be given over a 2-hour infusion period and that the treatment course may be shortened to 5 days [15]. On the basis of this study, we administered the drug at a dose of 0.12 mg/kg daily in 2-hour intravenous infusions for 5 consecutive days in the majority of our studies [16-20]. This route of administration is free from inconvenience, usually associated with continuous infusion and rarely induces thrombotic complications. Moreover, the 2-hour infusion may be given on an outpatient basis. 2-CdA can also be administered in subcutaneous injections and orally [21,22]. These routes result in substantial improvement of the quality of life in disorders that require repeated courses of treatment such as chronic lymphocytic leukemia (CLL) and non-Hodgkin's lymphoma

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(NHL). Unfortunately, data from randomized controlled trials regarding continuous versus intermittent or 5 day versus 7 –day protocols are not available.

2-CdA was approved by the FDA for treatment of HCL and in some European countries for treatment of refractory/relapsed CLL. Recently however, several clinical trials have been undertaken to confirm the value of this agent in other indolent lymphoid malignancies including low grade NHL (LG-NHL) and Waldenström macroglobulinemia (WM). 2-CdA has been also investigated in patients with acute myeloid leukemia (AML), especially in combination with standard treatment. Moreover, some clinical data suggest that 2-CdA may have some value in multiple sclerosis (MS) and other autoimmune disorders. Recent clinical trials, evaluating the activity of 2-CdA used alone or in combination with other agents in the treatment of hematological malignancies and autoimmune diseases are the subject of this review.

Hairy Cell Leukemia

Hairy cell leukemia (HCL) is a distinct B-cell lymphoproliferative disorder with clearly defined diagnostic criteria, usually characterized by anemia, granulocytopenia,

monocytopenia, thrombocytopenia and splenomegaly [23]. Treatment of HCL should be considered for symptomatic patients. The new purine analogs, 2-CdA and pentostatin (DCF), constitute a novel group of agents with high activity in HCL [24-27]. In 1987 Piro *et al.* [28] first described two patients with HCL who had undergone splenectomy and each received a single continuous intravenous infusion of 2-CdA for 7 days. Both patients had sustained complete responses (CR).

Further multiple studies on larger groups of patients have demonstrated that 2-CdA induces durable and unmaintained CR in about 80% of patients after a single course of therapy [24-27]. 2-CdA was administered either as a continuous i.v. infusion at a dose of 0.09 mg/kg over a 5-7 day period or as 2-hour i.v. infusion at a dose of 0.12 mg/kg for 5-7 days. However, similar results have been achieved when the drug was given in subcutaneous injections [29].

Preliminary observations indicated that a CR following 2-CdA administration was durable even without maintenance therapy, so this drug was considered as potentially curative against HCL [30,31]. However, a long-term clinical follow-up of patients who entered CR also after a single course of 2-CdA revealed that about 20% of them relapsed (Table 1)

Table 1. Summary of larger clinical trials of 2-CdA in hairy cell leukemia

Authors and study design	Treatment	No of pts	CR rate (%)	Duration of CR (median time to relapse)	Rate of relapse at follow-up
Saven <i>et al.</i> 1998 [38] Prospective	c.i.	349	91	96% survival at 48 months	26% at median 29 months
Goodman <i>et al.</i> 2003 [33] Prospective	c.i.	207	95	98 months	37% at median 44 months
Hoffman <i>et al.</i> 1997 [35] Prospective	c.i.	49	76	RFS 80% at median 55 months follow-up	NA
Cheson <i>et al.</i> 1998 [25] Prospective, multicenter	c.i.	861	50	Median not reached	NA
Robak <i>et al.</i> 1996 [27] Retrospective, multicenter	2 hour i.v. infusion/5 days or c.i. /7 days	41	76%	NA	NA
Jehn <i>et al.</i> 2004 [30] Prospective	c.i.	44	98%	DSF 36% at 12 years OS 79% at 12 years	NA
Von Rohr <i>et al.</i> 2002 [29] Multicenter, Phase II	s.c. bolus injection/5 days	62	76%	NA	NA
Lauria <i>et al.</i> 1999 [40] Prospective, phase II	0.15 mg/kg weekly for 6 weeks	25	76%	NA	NA
Robak <i>et al.</i> 1999 [37] Prospective, multicenter	2 hour i.v. infusion / 5 days	97	77.3%	37.4 months	26.7%
Chadha <i>et al.</i> 2005 [24] Prospective, phase II	0.1 mg/kg/day x 7,ci	86	79%	RFS 54% at 12 years	
Zinzani <i>et al.</i> 2004 [39] Retrospective	0.14mg/kg/d for 5d or once a week for 5 cycles	37	81%	27% at 122 months	52% at 13 y
Juliusson <i>et al.</i> 1995 [43] Prospective, multicenter	s.c. injections for 7 days	73	81%	NA	NA

Abbreviations: CR-complete response, DFS – disease free survival, OS –overall survival RFS – relapse free survival, 2-CdA – 2-chlorodeoxyadenosine, NA - not applicable, i.v. –intravenous, s.c.- subcutaneous, c.i. – continuous infusion; d-day; yr –year.

[32-39]. Patients in apparent clinical and hematological remission following a single course of 2-CdA administration may have the residual disease detected with the use of flow cytometry or molecular assay [26,36]. These data seem to indicate that a single course of 2-CdA may not be sufficient to eliminate the entire leukemic clone.

2-CdA is also an effective drug when administered at a dose of 0.15 mg/kg in 2-hour infusion once a week over 6 courses. In the study by Lauria *et al.* [40] 22/30 (73%) patients with HCL achieved complete response (CR) and 8 (27%) – partial response (PR) when 2-CdA was given in this mode. This type of drug administration may be less toxic and reduces the risk of infection complications in comparison with standard 2-CdA daily regimens. In our randomized study we compared weekly administration of 2-CdA (0.12 mg/kg in 2-h i.v. infusion once a week for 6 weeks) with daily administration (0.12 mg/kg in 2-h i.v. infusion for 5 consecutive days) [41]. The updated results of this study indicate that both CR and overall response (OR) rates were similar in compared groups (Table 2). There was no statistically significant difference in toxicity between groups except for thrombocytopenia. It seems, however, that daily administration of 2-CdA may more frequently induce thrombocytopenia and neutropenia and lead to more frequent infections.

The study of Liliemark *et al.* [42] has shown that 2-CdA can be also administered in subcutaneous injections. It has been shown that there is 100% 2-CdA bioavailability after subcutaneous administration with high peak concentrations of short duration in the area under the curve (AUC) very similar to those achieved with 1-hour intravenous infusions. After the subcutaneous dose of 3.4 mg/m² body surface area, the median AUC in plasma following first injections was 567.5 µmol/L/h. This value is similar to the corresponding data achieved with intravenous continuous or 2-hour bolus infusions. The median half-life of 2-CdA in plasma was 7.9 hours (range 3.6-39.8) following subcutaneous injection as compared to 9.9±4.6 hours following bolus infusions [42]. It should be noted that there were no local side effects when using the subcutaneous route.

In the nonrandomized, phase II study of Juliusson *et al.* [43] 73 patients were given 2-CdA as a subcutaneous injection once daily for 7 days. Fifty-nine patients (81%) achieved a durable CR after one (n=55) or two courses and 10 had a PR. With a median follow-up duration of 20 months

no patient had a clinical relapse. Similar study was performed by von Rohr *et al.* [29]. In this multicenter phase II trial 62 patients received a first cycle with 2-CdA at a dose of 0.14 mg/kg/day by subcutaneous bolus injection for 5 consecutive days. Complete and partial responses were seen in 47 (76%) and 13 (21%) patients, respectively. Most responses occurred within 10 weeks after starting 2-CdA therapy. At a median follow-up of 3.8 years, progression after PR was seen in 7 patients and relapse after CR was seen in 8 patients. The above two trials indicate that 2-CdA given by subcutaneous bolus injections is very effective in HCL and more convenient for patients than continuous intravenous infusion. However, randomized, phase III study comparing intravenous versus subcutaneous administration of 2-CdA in HCL has not been performed so far.

Chronic Lymphocytic Leukemia

CLL is a clonal hematopoietic disorder characterized by proliferation and accumulation of small lymphocytes. CLL is the most common adult leukemia in Europe and Northern America with an annual incidence rate of three to five cases per 100 000 [44,45]. The median age at diagnosis is about 65 years with only 10-15% of patients under the age of 55 years. The diagnosis of CLL does not imply the need for immediate therapy and the management of CLL patients is determined by the stage and activity of the disease.

The activity of 2-CdA in patients with CLL resistant to conventional treatment was first reported in 1988 by Piro *et al.* [46]. Out of 18 patients an overall response was achieved in 10. The same group reported later a response rate of 44% in 90 patients with advanced and previously treated CLL [47]. However, only 4% of the patients achieved CR. In this study, 2-CdA course consisted of 0.05 to 0.2 mg/kg/d for seven days by continuous infusion. Patients were given between one and four courses of 2-CdA and responses were achieved after an average of 2 courses of therapy. Similar results have been reported by others (Table 3) [46-53]. In our study of 184 patients with CLL in relapse or refractory to previous therapy we found an overall response rate of 48.4% including 12.5% CR [30]. 2-CdA was administered at a dose of 0.12 mg/kg/daily in 2-hour intravenous infusions for 5 consecutive days. Similar results were achieved by Betticher and colleagues when the drug was administered subcutaneously [22]. In this study patients were treated with 2-CdA given at a reduced dose of 0.6 mg/kg/cycle as s.c bolus injections for five days. The overall response (OR) rate

Table 2. Randomized comparison of weekly administration and daily courses of 2-CdA in patients with hairy cell leukemia (adapted from [41])

Patients	Number of patients	CR	OR	Thrombocytopenia Gr 3/4	Neutropenia Gr 3/4	Infections Gr 3/4	Relapsed	Died
Daily administration	59	43 (73%)	50 (85%)	8 (13.6%)	21 (35.6%)	14 (24%)	5 (8.5%)	6 (10.5%)
Weekly administration	59	44 (76%)	49 (85%)	2 (3.4%)	12 (20.3%)	6 (10.2%)	5 (8.5%)	4 (6.8%)
p value		0.8	0.8	0.04	0.07	0.05	1.0	0.5

Abbreviations: CR – complete response; OR – overall response; Gr – grade.

Table 3. 2-CdA in previously treated patients with CLL

Authors and Study Design	Number of patients	OR (%)	CR (%)	Median duration of response (months)
Saven <i>et al.</i> [47] Prospective, phase II	90	44	4	4
Tallman <i>et al.</i> [49] Prospective, phase II	26	31	0	16
Robak <i>et al.</i> [52] Retrospective	92	36	5	NA
Juliusson <i>et al.</i> [50] Prospective, phase II	52	58	31	20
Rondelli <i>et al.</i> [51] Prospective, phase II	19	68	11	CR 9+
Robak <i>et al.</i> [48] Prospective, multicenter	184	48.4	12.5	10
Betticher <i>et al.</i> [53] Prospective, phase II	55	38	5	6

Abbreviations: NA – data not available; OR – overall remission rate; CR – complete remission rate.

was 40% and was similar to that in a group of 20 patients treated with 2-CdA at a dose of 0.7 mg/kg/cycle as continuous i.v. infusion for 7 days. Moreover, dose reduction by 29% resulted in significant decrease of myelotoxicity and risk of infection.

Despite the high response rate the influence of 2-CdA on survival duration in CLL is still uncertain. However, in our retrospective analysis we found that patients who received high dose chlorambucil with prednisone as a front-line therapy followed by 2-CdA with or without prednisone as a second line treatment survived significantly longer than the patients treated with lower doses of chlorambucil as first line and COP (cyclophosphamide, vincristine, prednisone) and/or

CHOP (COP + doxorubicin) as second line treatment and never treated with 2-CdA [54]. It should be underlined that the difference in survival was seen only in patients with more advanced clinical stages of CLL (Rai III and IV), whereas in less advanced stages (Rai 0, I, II) survival was similar in both groups.

Clinical stages of CLL are among the most useful prognostic factors. In our study we found greater overall response rate in patients in stage I and II (62.5%) than in stage III and IV (42.2%) ($p=0.01$) according to Rai's classification [48]. There was also significant difference in survival time of both groups (15.0 and 10.0 months respectively; $p=0.0001$) (Fig. 1).

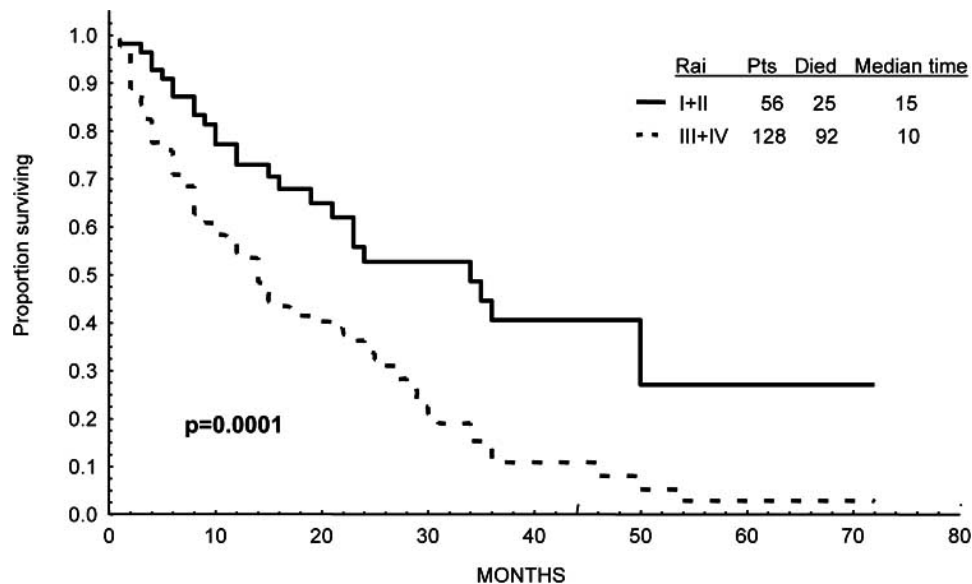


Fig. (1). Survival of pretreated B-CLL patients treated with cladribine (2-CdA) by Rai stage evaluated from the beginning of 2-CdA treatment (modified from [48]).

2-CdA has been found to be more effective in previously untreated CLL than in patients refractory to or relapsed after conventional therapy with alkylating agents. In different studies the overall response (OR) rate ranged from 75 to 85% and complete response (CR) from 10 to 47% (Table 4) [48,53-59]. In our phase II study on 194 previously untreated patients with CLL, CR was observed in 88 (45.4%) patients and PR in 72(37.1%) for an overall response rate of 82.5% [48]. The median duration of OR (CR or PR) in this group of patients was 12.0 months and that of CR 13 months. The

median survival was longer in patients who responded to 2-CdA treatment than non-responders (Fig. 2).

The effectiveness and toxicity of the oral formulation of 2-CdA in CLL patients has been also investigated [55,57]. Juliusson *et al.* [57] treated 63 patients with symptomatic but previously untreated CLL with 2-CdA solution 10 mg/m²/d orally for 5 consecutive days in monthly courses. OR was achieved in 47 (75%) patients including CR in 24 (38%) patients. The median response duration was not reached at 2

Table 4. Activity of 2-CdA as first-line treatment in patients with CLL

Authors and study design	Number of patients	Method of administration	OR (%)	CR (%)	Median duration of response (months)
Saven <i>et al.</i> [56] Prospective, phase II	20	0.1 mg/kg/d c.i.v. for 7 days every 28;35 d	85	10	8+
Juliusson <i>et al.</i> [57] Prospective, phase II	63	10 mg/m ² /d po for 5 days, monthly	75	10	14
Tallman <i>et al.</i> [58] Prospective, phase II	54	0.14 mg/kg/d 2-h i.v. every 28 d	81	26	NA
Delannoy <i>et al.</i> [59] Prospective, phase II	19	0.12 mg/kg/d 7, 2-h i.v. monthly	74	47	NA
Robak <i>et al.</i> [48] Prospective, multicenter phase II	194	0.12 mg/kg/d x5. 2-h i.v. every 28d	82.5	45	12.0
Karlson <i>et al.</i> [21] Prospective, multicenter, phase II	61	10mg/m ² /d, po for 3d every 3 weeks	81	15	20

Abbreviations: CLL - chronic lymphocytic leukemia; OR - overall response; CR - complete response; c.i.v. -continuous intravenous infusion; po - orally; i.v. - intravenous infusion; d - day; NA - data not available.

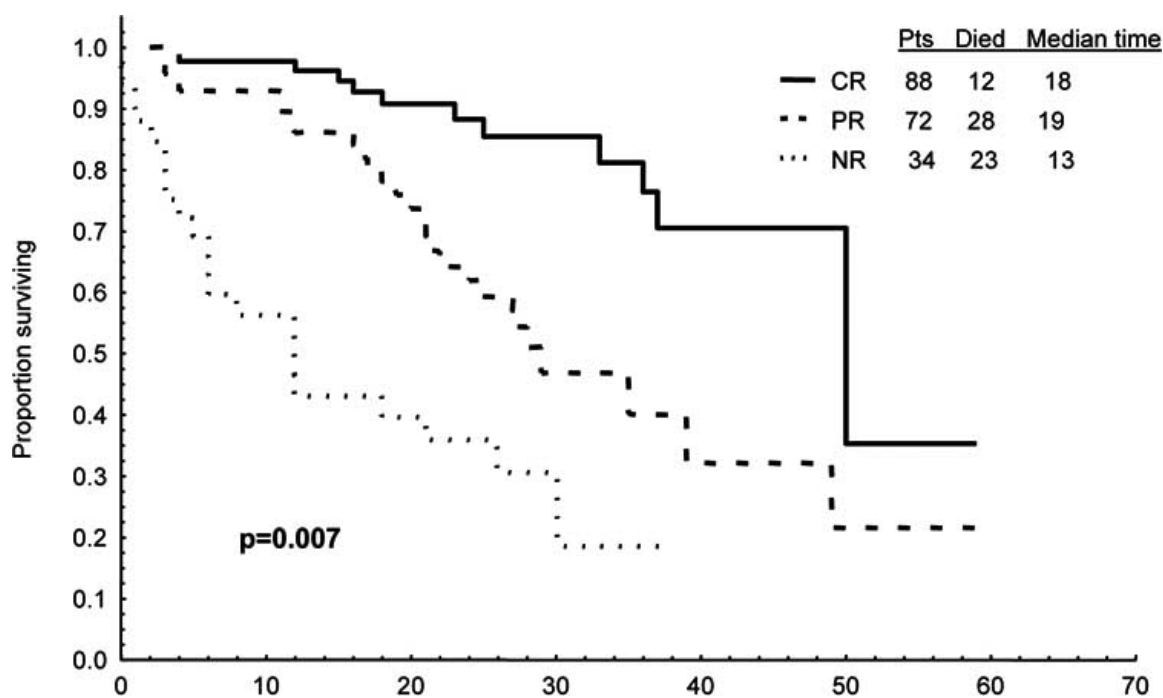


Fig. (2). Survival of previously untreated B-CLL patients treated with cladribine (2-CdA) by response evaluated from the beginning of 2-CdA treatment (modified from [48]).

years and the overall survival rate at 2 years was 82%. However, grade 3/4 infections toxicity occurred in 22 (35%) patients. More recently, the same group presented the results of a phase II trial of oral 2-CdA with a 3-d, 3-weekly schedule in untreated and pretreated patients [55]. In the group of 63 previously untreated patients OR was achieved in 81% including CR in 20% of the patients. The results of previously untreated patients were retrospectively compared with 63 patients from previous study [57] using a 5-d monthly schedule. The oral dose corresponded to 5 mg/m² by the intravenous route. No significant difference regarding the response in these two cohorts of patients was observed. The above study demonstrates that oral 2-CdA has efficacy and safety similar to the intravenous formulation. Unfortunately oral formulation of this agent is not commercially available yet.

High CR and OR rates in CLL patients treated with 2-CdA as a first-line therapy were confirmed in a multicenter prospective, randomized trial. In this study we compared the efficacy and toxicity of 2-CdA with prednisone and chlorambucil with prednisone in previously untreated patients with progressive and advanced CLL [60]. 2-CdA was administered at a dose of 0.12 mg/kg daily in 2-hour infusion for 5 consecutive days and was combined with oral prednisone 30 mg/m² daily on days 1 to 5 starting with 2-CdA courses. Chlorambucil was administered at a dose of 12 mg/m² per day for 7 consecutive days and prednisone was given at a dose of 30 mg/m² per day on days 1 to 7. Both cycles were repeated at monthly intervals or longer if hematologic complications or severe infections developed. Guidelines for response evaluation were those developed by the NCI – Sponsored Working Group [61]. Evaluation of response was performed after 3 courses. Treatment was discontinued if CR was achieved. If there was partial

response (PR), up to three additional courses were administered. Patients without response after three courses or who had relapsed earlier than 12 months after completing chemotherapy were switched to the alternative arm. Patients who relapsed 12 months or later from achieving the remission were retreated with the same protocol that induced the previous response. Of 229 evaluated patients 126 received 2-CdA with prednisone and 103 received chlorambucil with prednisone as first line treatment. Data obtained from our trial indicate that the OR rate after 2-CdA and prednisone was significantly higher than after chlorambucil and prednisone (87 and 57%, respectively, $p < 0.001$). The CR after 2-CdA and prednisone was also significantly higher than after chlorambucil and prednisone (47 and 12%, respectively, $p < 0.001$). Moreover, progression-free survival (PFS) was significantly longer in the 2-CdA treated group ($p = 0.01$) (Fig. 3). The probability of PFS calculated from Kaplan-Meier curves at 24 months for patients who received 2-CdA with prednisone or chlorambucil with prednisone were 46% and 33%, respectively ($p = 0.01$). The important part of our study was the evaluation of event-free survival (EFS) and overall survival (OS). EFS was defined as the time from the beginning of first-line treatment to the first adverse event (death, progression requiring a change in therapy, infections or thrombocytopenia, hemorrhage requiring hospitalization and autoimmune hemolytic anemia). EFS was similar in both groups of patients (log-rank test, $p = 0.09$) (Fig. 4). At the time of analysis, death rates have also been similar in patients treated with 2-CdA (20%) and with chlorambucil (17%). The updated probability of overall survival calculated from Kaplan-Meier curves was also similar for both groups (Fig. 5). However, it should be emphasized that, our trial was designed as a cross-over study and most patients in the chlorambucil group were treated with 2-CdA, as the second-

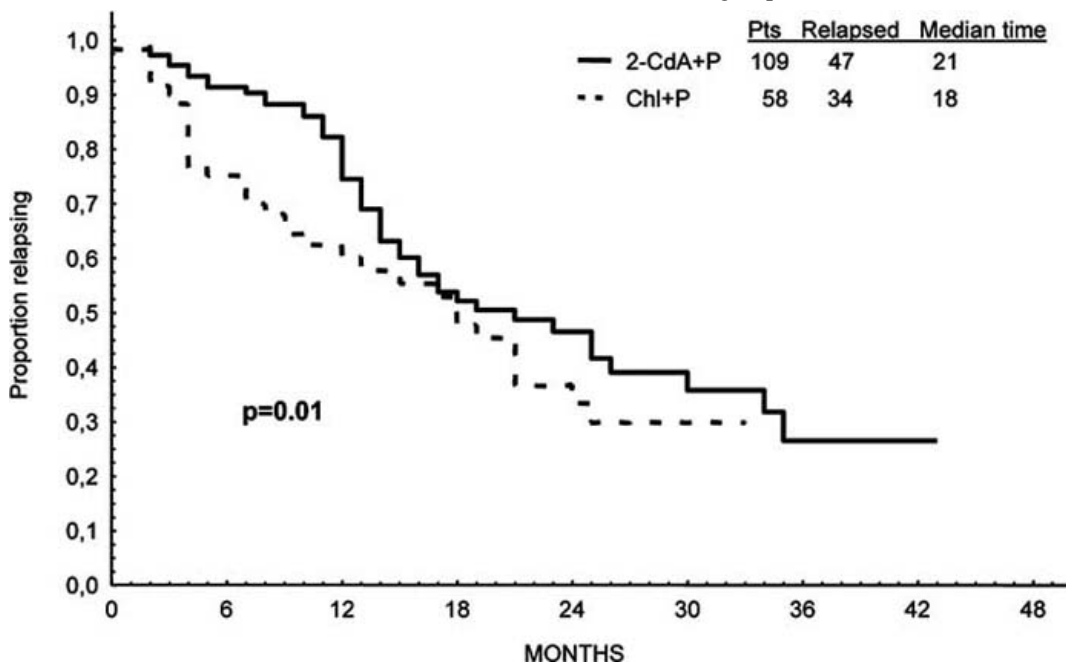


Fig. (3). Progression free survival defined as the time from the end of the first-line therapy to disease progression or death for CLL patients in CR or PR after treatment with cladribine (2-CdA)+Prednisone (P) (continuous line) or chlorambucil (Chl+P) (dotted line) (modified from [60]).

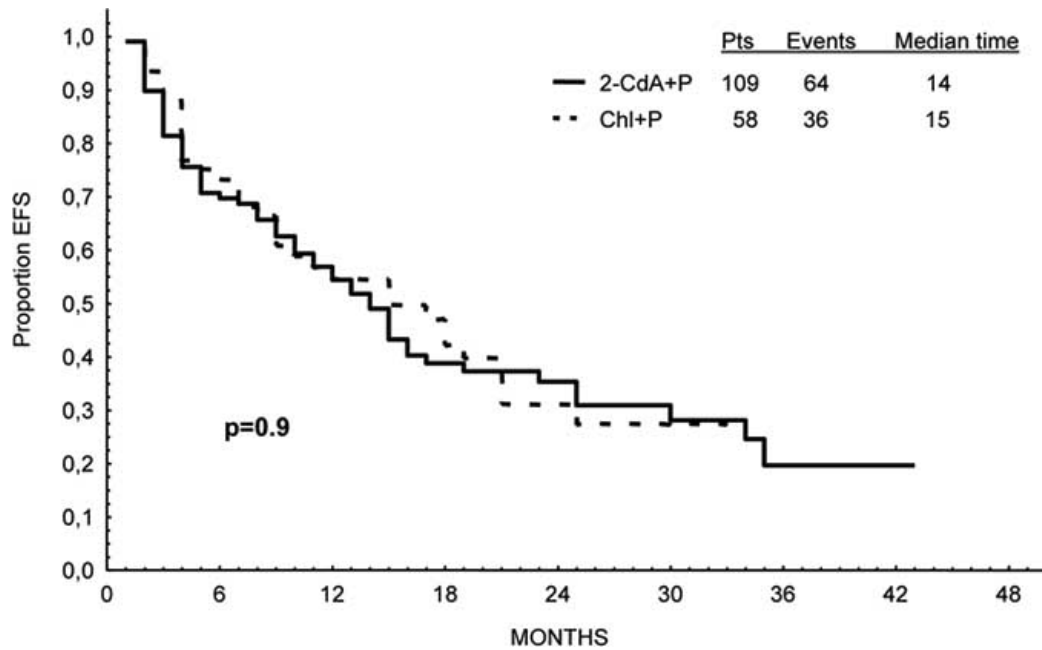


Fig. (4). Event free survival (EFS) defined as the time from the beginning of the first-line therapy to a first adverse event: death, progression requiring a change in therapy, infections or thrombocytopenic hemorrhage requiring hospitalization and autoimmune hemolytic anemia for the patients who responded to the first line treatment with cladribine (2-CdA)+Prednisone (P) (continuous line) or chlorambucil (Chl+P) (dotted line) (modified from [60]).

line therapy in case of refractory disease or early relapse [60].

An international, randomized study to compare 2-CdA, fludarabine (FA) and chlorambucil in previously untreated patients with CLL has been initiated by Karlson *et al.* [62]. Preliminary results of this multicenter study show that both efficacy and toxicity of 2-CdA and FA in first-line treatment are similar. The study comprised 150 patients and 139 were

evaluated. Overall response according to NCI criteria and intention to treat were documented in 57% of patients in chlorambucil group, 67% in FA group and 74% in 2-CdA group. Myelotoxicity and infections also did not differ between all three arms.

Recently, we have performed an interim analysis of randomized study comparing activity and toxicity of 2-CdA and cyclophosphamide (CY) (CC program) versus FA and

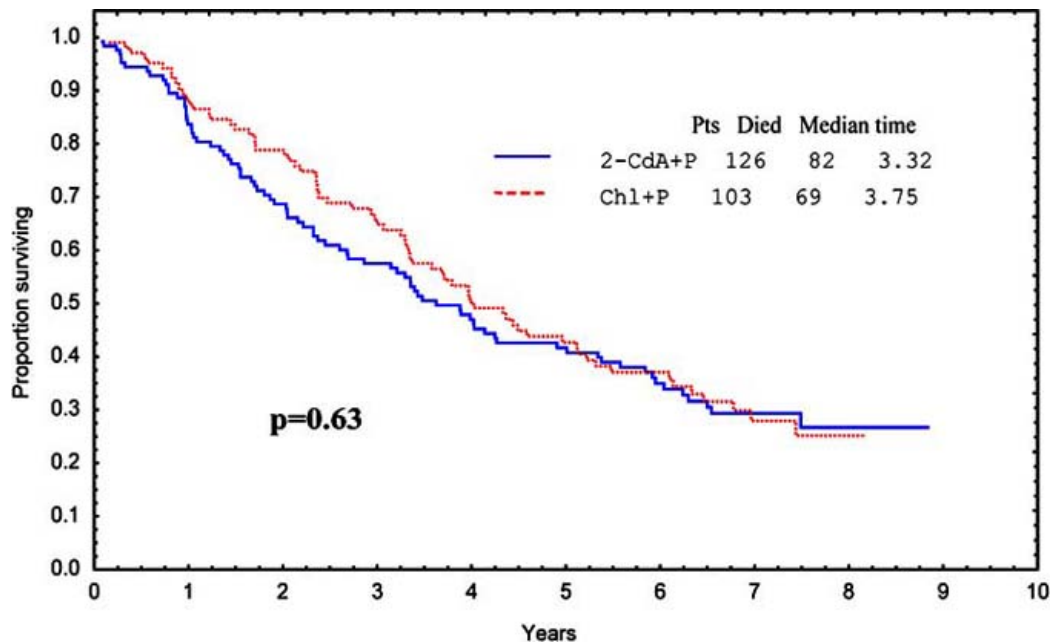


Fig. (5). Overall survival time calculated from the first day of 1st line treatment to the last day of follow-up or death for patients treated with 2-CdA+P (continuous line) or Chl+P (dotted line) in randomized, multicenter study. Modified and updated from [60].

CY (FC program) in previously untreated progressive or symptomatic CLL [63]. The preliminary results of this study indicate that CC and FC programs used as first-line therapy gave similar CR (36.7% vs. 56.3%, $p=0.1$) and OR (93.3% vs 87.5%, $p=0.37$) and comparable toxicity. (Table 5).

The recent data indicate that 2-CdA administered in combination with other chemotherapeutic agents and/or monoclonal antibodies (MoAb) may produce higher response rates including CR and molecular CR compared with 2-CdA in monotherapy or other treatment regimens.

In 1993 we demonstrated synergistic action of 2-CdA and CY on murine leukemias L1210 and P388 [64]. Furthermore, Van Den Neste *et al.* [65] have shown recently in the *in vitro* study that 2-CdA potentiates antitumor effects of CY derivatives on B-CLL cells. The results of these experiments were confirmed by the clinical studies combining 2-CdA with CY that have demonstrated significant activity in previously untreated and pretreated patients with CLL [66-68].

Montillo *et al.* [67] treated 29 patients with refractory or recurrent CLL or prolymphocytic leukemia (PLL) with 2-CdA (4 mg/m²/day) and CY (350 mg/m²/day) for 3 days every 4 weeks. Eleven patients (38%) had a response with median response duration of 12 months. Neutropenia was the most common form of hematologic toxicity and was observed after 25% of 84 courses. We determined the effectiveness and toxicity of 2-CdA in combination with CY in patients with previously untreated CLL [68]. 2-CdA was administered at a dosage of 0.12 mg/kg for 3 consecutive days and CY at a dose of 650/mg/m² on day 1. The cycles were repeated at four-week intervals or longer if severe myelosuppression occurred. In the analyzed group of 82 patients the OR rate of 87.8% was achieved including CR in 29.3% of patients, MRD was only detected in six out 24 (25%) patients with CR. Grade 3/4 thrombocytopenia was seen in four patients (5%) and neutropenia in 10 (12%). Severe infections were noted in 21 (25%) patients. The efficacy of this regimen seems to be higher than observed earlier after treatment with 2-CdA alone and similar to the results of FA combined with CY.

Subsequently we determined the effectiveness and toxicity of combined chemotherapy consisting of 2-CdA, mitoxantrone (MIT) and CY (CMC regimen) in the

treatment of refractory or relapsed indolent lymphoproliferative disorders [69, 70]. The treatment course consisted of 2-CdA at a dose of 0.12 mg/kg/day in 2-h intravenous infusion for 5 (CMC5) or 3 (CMC3) consecutive days, MIT 10 mg/m² i.v. on day 1 and CY 650 mg/m² i.v. on day 1. The overall response rate was 48.6%. There was no difference in the frequency of response between the CMC3 and CMC5 treated groups. However, infections and fever of unknown origin complicated the treatment with CMC5 more often than with CMC3. The CMC program is an active combined regimen both in pretreated and therapy naive CLL patients. Recently, we performed a randomized multicenter study to compare the CMC3 regimen with 2-CdA alone and in combination with CY (CC program) [71]. All regimens were repeated every 28 days or at longer intervals if hematologic complications occurred. The minimal residual disease (MRD) was evaluated by flow cytometry. The updated results are summarized in Table 6 and seem to indicate that CMC program used as first-line therapy gives higher CR rate (39.6%) than 2-CdA alone (25.9%) or 2-CdA combined with CY (28.3%) ($p=0.03$). However, OR rate was similar in all three groups (79.1%, 74.1%, 82.2% respectively, $p=0.1$). Moreover, 2-CdA and CC programs were less myelotoxic than CMC. In our *in vitro* studies on fresh CLL cells we have shown that the combination of rituximab (RIT) with 2-CdA induces the additive pro-apoptotic effect [72]. In the clinical study we investigated the efficacy and toxicity of combined therapy consisting of RIT and 2-CdA (RC regimen) in patients with refractory or relapsed indolent lymphoproliferative disorders [73]. RC regimen consisted of RIT at a dose of 375 mg/m² in 6-hour infusion on day 1 and 2-CdA at a dose of 0.12 mg/kg in 2-hour infusion given on days 2-6. The RC courses were repeated at 4-week intervals or longer if severe myelosuppression occurred. Twenty-six patients, 15 with CLL and 11 with LG-NHL, were enrolled into the study. All patients received 5 or more cycles of chemotherapy before RC treatment. Twelve patients were recurrent after prior chemotherapy and 14 were refractory. The median number of RC courses was 3, range 1-5. Eighteen patients responded, 69% in LG-NHL and 73% in CLL group. Four patients achieved a CR and 14 a PR. The remaining 8 patients had either a stable disease or progressed. There was no statistical difference in FFS between CLL and LG-NHL patients. The median FFS for responders was 6.5 months. There was a significant

Table 5. Cladribine with cyclophosphamide vs fludarabine with cyclophosphamide as first-line treatment in chronic lymphocytic leukemia: an early report of prospective, randomized study (PALG CLL3) (adapted from [63])

Treatment	Patients n=172		OR (%)	CR (%)	Thrombocytopenia Gr 3/4	Neutropenia Gr 3/4	Infections	Died
	Enrolled	Evaluated						
CC	87	30	28 93.3%	11 36.7%	5 16.7%	11 36.7%	21 70%	2 6.7%
FC	85	32	28 87.5%	18 56.3%	3 9.4%	10 31.2%	16 50%	2 6.2%
P value			0.37	0.1	0.32	0.43	0.09	0.67

Abbreviations: CC – cladribine + cyclophosphamide; FC – fludarabine + cyclophosphamide; OR – overall response; CR – complete response.

Table 6. Randomized comparison of 2-CdA alone and in combination with cyclophosphamide or cyclophosphamide and mitoxantrone as first line treatment in patients with chronic lymphocytic leukemia (adapted from [71])

Treatment	2-CdA	CC	CMC	p value
Entered patients	167	169	163	
Evaluated patients	143	152	139	
CR	37 (25.9%)	43 (28.3%)	55 (39.6%)	0.03
OR	106 (74.1%)	125 (82.2%)	110 (79.1%)	0.2
Median OR duration (years)	1.67	1.81	1.43	0.1
Relapse	48 (45.3%)	45 (29.6%)	43 (30.9%)	0.2
AIHA	10 (7.0%)	10 (6.6%)	5 (3.6%)	0.4
Thrombocytopenia gr III/IV	25 (17.5%)	25 (16.4%)	32 (23.0%)	0.3
Neutropenia gr III/IV	27 (18.9%)	43 (30.9%)	52 (37.4%)	0.01
Infections	39 (27.3%)	47 (30.9%)	53 (38.1%)	0.07
Died	39 (27.3%)	30 (19.7%)	37 (26.6%)	0.4

Abbreviations: 2-CdA- cladribine; CC -2-CdA+cyclophosphamide; CMC-2 - CdA + clophosphamide + mitoxantrone; CR – complete response; OR – overall response; AIHA – autoimmune hemolytic anemia.

difference in OS between responders and non-responders. Severe neutropenia (grade 3) was seen in 3 patients, thrombocytopenia was only grade I/II and was observed in 2 patients. Hypersensitivity to RIT with fever, chills, and hypotension was the major toxicity of RC regimen and occurred in 9 patients. It was mostly observed during the first cycle. In only one case the treatment was stopped after the first cycle because of severe allergic reaction during the second administration of RIT. Four episodes of grade 3/4 infections were also observed. There was no treatment-related mortality.

Taking into account the observation that the combination of 2-CdA with cyclophosphamide (CC regimen) may be more effective than 2-CdA alone, the combined use of RIT with CC regimens is an attractive option. Recently, we have designed the RCC protocol in which RIT is administered at a dose of 375 mg/m² on day 1, 2-CdA at a dose 0.12 mg/m²/d in 2h i.v. infusions is given on days 2-4 and cyclophosphamide is administered at a dose of 250 mg/m² i.v. also on days 2-4. Courses are repeated at 28 day intervals or longer if myelosuppression and/or infection develop for a maximum of 6 courses. The preliminary results of our current study may also indicate that the combination of 2-CdA with CC (RCC) regimen is more effective than RC program.

Finally, several phase II and III trials support the use of 2-CdA alone or in combination in patients with CLL. However, this drug is not used as front – line therapy in most institution, and only in few European countries this agent is registered for use for this indication. In several centers alkylating agent, chlorambucil, is still the drug of choice in first line therapy. As the majority of patients are older and since there is no survival time advantage for either alkylating agents and fludarabine or 2-CdA therapies chlorambucil is safe, has a low incidence of therapy related toxicity, can be administered on an out patient basis, and most importantly, the cost involved in administering such treatment is low. However, purine nucleoside analogs, fludarabine or 2-CdA, should be used rather in younger patients. In this group of patients the rate and quality of CR evaluated by the estimation of residual disease may be of special importance. This favors the early use of fludarabine or 2-CdA.

Fludarabine, however in contrast to 2-CdA is registered for first line therapy in Western Europe.

Waldenström's Macroglobulinemia

Waldenström's macroglobulinemia (WM) is defined as a distinct chronic lymphoproliferative disorder with characteristic marrow morphology and phenotype and the presence of significant IgM paraprotein. In this disease 2-CdA has been shown to be active in 64% to 100% of previously untreated patients and in 14% to 78% of refractory or relapsed patients [74-81]. The number of cycles administered in these studies varied considerably but significant tumor reduction was observed as early as after two courses of 2-CdA. The median time to response ranged from 1.2 months to 5.8 months in various studies. The median time to response to 2-CdA in previously untreated patients varied between 13 and 28 months [82,83]. Longer responses were found in patients receiving more cycles of therapy. 2-CdA was also administered to patients in whom primary treatment with alkylating agents had failed. The rate of objective responses ranged from 14% to 53% in different studies [74,75,78,80]. The response rate was higher and the duration of response was longer when 2-CdA was given to patients with primary refractory disease rather than to patients with disease in resistant relapse [82].

In the majority of studies 2-CdA was administered in continuous intravenous infusion at a dose of 0.1 mg/kg/day for 7 days [74-76] or 0.12 -0.14 mg/kg/day in 2-hour infusion for 5 days [79,80]. However, in one study the drug was given as subcutaneous bolus injections at a dose of 0.1 mg/kg/day over 5 days [78]. Twenty-five previously treated refractory or relapsed patients received therapy every 4 weeks or at longer intervals for a maximum of 6 cycles. Overall response rate was 68% and median remission duration was 8 months. However, there are no comparative data to recommend the use of 2-CdA or FA as a reasonable choice if PNA therapy is being considered.

Other Indolent Lymphoid Malignancies

2-CdA showed remarkable activity in both previously treated and untreated LG-NHL. In relapsed/refractory LG-

NHL patients 2-CdA induced durable responses with OR rates range from 32 to 76% and CR rates between 10% and 38% (Table 7) [84-91]. 2-CdA was even more active in previously untreated patients [92-94]. OR rate was achieved in 64-88% and CR in 25-32%. 2-CdA was effective in combination with alkylating agents and MIT in the treatment of refractory or relapsed advanced stage LG-NHL [95-98]. The combination of 2-CdA with MIT and CY (CMC regimen) proved to be very active in heavily pretreated LG-NHL patients, however, due to its significant toxicity the period of 2-CdA administration was recommended to be reduced from 5 to 3 days [69].

Recently, lower doses of 2-CdA (5 mg/m²/week) have been investigated both in monotherapy [99] and in combination with MIT [100]. The results demonstrate that these reduced doses of 2-CdA are highly active and possibly better tolerated than standard doses in the treatment of indolent lymphoid malignancies.

Recently, interim analysis of randomized multicenter trial comparing 2-CdA alone with its combination with CY (CC) and standard combined regimen COP (CY, vincristine, prednisone) in previously untreated patients with LG-NHL has been presented [101]. The first interim analysis included 105 out of 165 patients randomized in 17 centers in Poland.

Compared to 2-CdA and CC, COP induced lower OR rates (75%, 85%, and 51% respectively, $p=0.005$) including CR rates (43%, 62.5% and 5.5% respectively, X^2 test $p<0.001$). With a median follow-up of 10 months median progression free survival (PFS) was superior in patients receiving 2-CdA, containing regimens (8 versus 11 versus 6 months, respectively; $p<0.001$). However, no difference in median OS was detected (9 versus 12 versus 7 months, respectively $p=0.56$). The first interim analysis has resulted in discontinuation of accrual in the COP arm.

2-CdA is structurally similar to FA. Both agents have been found to be more effective in previously untreated LG-NHL than in patients refractory to or relapsing after conventional therapy. Recently, one phase II randomized study performed on previously treated LG-NHL patients showed that 2-CdA and FA give similar response rate and duration [102]. Sixty patients with relapsed or refractory LG-NHL were randomized to initial treatment with either FA 25 mg/m² or 2-CdA 0.14 mg/kg, each for five consecutive days every four weeks. Upon treatment failure, eligible patients were crossed over to the other study drug. Overall responses were 68% with FA and 72% with 2-CdA, and CR - 48% and 38%, respectively. For responding patients actuarial 3-year PFS was 58% with FA and 52% with 2-CdA. Treatment with both agents was well tolerated. However, two patients (8%)

Table 7. Summary of clinical trials of 2-CdA as a single agent in low-grade non-Hodgkin's lymphoma

Authors and study design	No of pts	Previous treatment	OR (%)	CR (%)	Median response duration	OS
Kay <i>et al.</i> [84] Phase II	40	+	43	20	5m	NR
Betticher <i>et al.</i> [85] Multicenter, phase II	104	+	54	15	9m	NR
Liliemark <i>et al.</i> [86] Phase II	36	+	42	14	9m	16 m
Robak <i>et al.</i> [87] Multicenter, phase II	94	+	51	13	CR-12 m; PR-6m	NR
Tulpule <i>et al.</i> [88] Phase II	28	+	32	14	CR-12 m; PR-28 m	273 m
Ogura <i>et al.</i> [89] Multicenter, phase II	43	+	58	14	28 m	NR
Rummel <i>et al.</i> [90] Multicenter, phase II	66	+	76	38	23 m	72% at 48m
Kong <i>et al.</i> [91] Phase II	22	+	45	36	NR	28 m
Saven <i>et al.</i> [92] Phase II	28	-	82	32	10 m	60% at 48m
Fridrik <i>et al.</i> [93] Prospective, multicenter	50	-	88	28	51% after 21 m	85% after 92 wks
Liliemark <i>et al.</i> [94] Phase II	44	-	64	25	NR	Median not reached after 40 m

Abbreviations: OR- overall response rate; CR – complete response rate; OS –overall survival; NR- not reported; m- months; wks-weeks.

in the FA group and 15 patients (47%, $p=0.001$) in the 2-CdA group were taken of the study because of persistent hematological toxicity. Further studies are required to optimize 2-CdA schedule and dosage in order to analyze hematological toxicity with maintaining antitumor activity in patients with LG-NHL.

2-CdA showed some activity in advanced cutaneous T-cell lymphoma (CTCL) patients, including Sezary syndrome (SS) and mycosis fungicides. This drug produced 24% OR in CTCL with high incidence of septic complications and significant treatment related mortality [103,104]. Moreover, in the phase II study in relapsed or refractory adult T-cell leukemia/lymphoma conducted by Tobinai *et al.* [105], 2-CdA showed no effect in this disease and patient enrollment has been discontinued.

Acute Myeloid Leukemia

2-CdA as a single agent has been also investigated in patients with acute myeloid leukemia (AML), both in children and in adults (Table 8) [106-111]. The first report concerning 2-CdA administration in AML comes from pediatrics. Santana *et al.* [106] conducted a phase I study in 18 heavily pretreated patients with refractory or relapsed AML and 13 with acute lymphoblastic leukemia (ALL). 2-CdA was given as a continuous 5-day infusion at doses of 3-

10.7 mg/m²/day. At dose levels above 6.2 mg/m²/day significant oncolytic responses occurred in all patients. There was a significant correlation between both the responsiveness by cell type and dose of 2-CdA. More oncolytic responses were observed in AML than ALL patients. Complete remission (CR) was obtained in two AML patients treated at 5.2 and 10.7 mg/m²/d of 2-CdA, respectively. The only dose-limiting toxicity was myelosuppression. The maximal tolerated dose was established at the level of 8.9 mg/m²/day for 5 days. This dose was used in the phase II study, which included 17 relapsed or refractory pediatric patients with AML and 7 with ALL [107]. The overall response rate in AML was 59% with 8/17 patients (47%) of CR after one or two cycles of 2-CdA. The hematological toxicity with neutropenia and thrombocytopenia grade 3 or 4 according to the National Cancer Institute (NCI) was developed in 34 of the 36 courses of 2-CdA. However, there were no deaths due to toxicity. Recently the same group have published updated results of this study [108]. Seventy-three children with newly diagnosed primary AML and 20 children with secondary AML or myelodysplastic syndrome (MDS) were treated with one or two 5-day courses of 2-CdA (8.9 mg/m²/day) given by continuous infusion. In patients with primary AML assessed for response CR rate was 24% after one course and 40% after two courses of 2-CdA. The highest CR rate was observed in patients with FAB M5 type of AML (45% after

Table 8. Larger studies evaluating the efficacy of 2-CdA in AML

Authors and study design	2-CdA doses	Patients characteristics	No. of patients	Age (years)	Complete remission	Median duration of CR
Santana <i>et al.</i> 1994 [106, 107] Phase II	2-CdA 8.9 mg/m ² /d for 5d (CI)	Untreated	22	7 (0.6-18.9)	6 (27%)	NR
Krance <i>et al.</i> 2001 [108] Phase II	2-CdA 8.9 mg/m ² /d for 5d (CI))	Untreated primary AML	73	4.9 (0-18.8)	37(51%)	NR
Vahdat <i>et al.</i> 1994 [109]	2-CdA 5-21 mg/m ² /d for 5d (CI)	Relapsed/refractory	36	47 (14-84)	3 (8%)	3 m
Wrzesien-Kus <i>et al.</i> 2003 [120] Phase II	2-CdA 5 mg/m ² /d x5+Ara-C 2 g/m ² x5d+G-CSF 300 ug/d sc x 6	Relapsed/refractory	58	45(18-67)	29(50%)	17wks
Juliusson <i>et al.</i> 2001 [115] Phase II	2-CdA 5mg/m ² /dx 4+Ara-C 61 g/m ² /dx 4+IDA (10mg/m ² /dx2	Primary treatment	34	71(60.5-84.5)	24 (71%)	NR
Hołowiecki <i>et al.</i> 2004 [116, 117] Randomized, phase III	2-CdA 5 mg/m ² /d x5 Ara-C 200mg/m ² /d x7+ DNR 60 mg/m ² /d x 3	Untreated	200	45 (16-60)	144(72%)	NR
Wrzesien-Kus <i>et al.</i> 2005 [121] Phase II	2-CdA 5mg/m ² dx5+Ara-C 2g/m ² /d x5d+MIT 10mg/m ² d1+G-CSF 300 µg/d sc x 6	Relapsed/refractory	43	44(20-66)	21 (49%)	NR

Abbreviations:AML- acute myeloid leukemia; 2-CdA- 2-chlorodeoxyadenosine; Ara-C –cytarabine; IDA – idarubicin; DNR- daunorubicin; MIT - mitoxantrone; G-CSF -granulocyte-colony stimulating factor; CI - continuous infusion; d -day; wk-week; NR - not reported.

one course and 70.6% after two courses). In 7 of 14 patients with secondary AML or MDS partial remission (PR) was obtained after one course of 2-CdA. The agent was well tolerated and its toxicity was acceptable.

Unfortunately, the encouraging results with 2-CdA in childhood AML have not been confirmed in adults [109,111-113]. Vahdat *et al.* [109] reported their preliminary results of the treatment with 2-CdA in 30 adult patients with relapsed AML. 2-CdA was administered at a dose of 5-21 mg/m²/day in continuous infusion for 5 days. The maximal tolerated dose was established at the level of 17 mg/m²/day for 5 days. CR was achieved in three patients. These responses persisted for 3, 2, and 3 months, respectively. The most significant adverse event was the development of a progressive sensorimotor peripheral neuropathy. The other side effects included prolonged myelosuppression, reactivation of a posttransplant Epstein-Barr virus-related lymphoma in one patient and tumor lysis syndrome in three of them.

In the phase II study of 2-CdA in adult patients with relapsed or refractory AML (median 60 years) performed by ECOG the drug was administered in 15 patients at a dose of 17 mg/m²/day for 5 days [110]. The second course of therapy was given to patients who had not achieved aplasia by day 21. 2-CdA was well tolerated. Prolonged pancytopenia with bone marrow hypoplasia occurred in nearly all patients. However, there was no CR, though in eight patients bone marrow aplasia was observed.

The results presented above indicate that 2-CdA used as a single agent is an active drug in children with AML, even with relapsed or refractory disease. However, this agent has little activity in adult patients with relapsed or refractory disease. In the study performed by Van den Neste *et al.* [111] the efficacy of 2-CdA at a dose of 0.1 for 7 days given with daunorubicin (DNR) at a dose of 50 mg/m²/day on days 5, 6, and 7 was evaluated in 14 relapsed or refractory adult AML patients. No CR was achieved and only one patient had a PR. Compared to 2-CdA alone, the addition of DNR to 2-CdA changed neither the response rate nor the toxicity. These unsatisfactory results can be explained at least in part by the study population. The relapsed patients had very short first CR duration, more than 36% had refractory leukemia and all the patients were previously treated with anthracyclines. Moreover, the patients were older (median age 57 years), and only 5% had good prognosis karyotypic abnormalities.

In vitro and *in vivo* pharmacological studies performed to determine the effect of pretreatment with 2-CdA on Ara-CTP accumulation in leukemic blasts demonstrated a 50-65% increase in the rate of Ara-CTP accumulation [112,113]. Basing on these observations, Kornblau *et al.* [113] treated 17 relapsed patients (15 with AML and 2 with MDS) with 2-CdA at a dose of 12 mg/m²/day and Ara-C at a dose of 1g/m² over 2-hour infusion/day, for 5 consecutive days. Two patients with AML achieved CR lasting 10 and 17 weeks, respectively. To identify the optimal schedule for infusion of Ara-C Crews *et al.* [114] randomized 49 pediatric patients with newly diagnosed primary AML to a 5-day induction course of 2-CdA 9 mg/m² and Ara-C 500 mg/m² given as either a 2-hour (arm A) or a continuous infusion (arm B). CR rate was significantly higher in arm B (63%) compared with arm A (42%) (p=0,045). However, no schedule-dependent

differences in the increase of intracellular Ara-CTP accumulation were observed. Juliusson *et al.* [115] studied 2-CdA with high dose Ara-C (HDAC) or HDAC and IDA. In the group treated with IDA six out of eight patients (75%) achieved CR without excessive toxicity. However, such promising results with a triple drug regimen were obtained in a relatively young and good-prognosis population of adult AML patients.

The results of most numerous randomized study, which evaluated the efficacy and toxicity of 2-CdA combined with Ara-C and DNR (DAC-7) has been recently published [116]. A total of 400 previously untreated AML patients (< 60 years) were randomized either to the DAC-7 or to the DA-7 regimen. DAC-7 protocol consists of DNR 60 mg/m²/day on days 1-3, Ara-C 200 mg/m²/day on days 1-7 and 2-CdA 5 mg/m² in 2-hour infusion on days 1-5. In DA-7 therapy the patients received the same doses of Ara-C and DNR but without 2-CdA. The overall CR rate equaled 72% for DAC-7 and 69% for DA-7 arm (p= NS). After a single course of DAC-7 induction, the CR rate equaled 64% and was significantly higher compared to 47% in the DA-7 arm (p=0.0009). Median hospitalization time during the induction was 7 days shorter for DAC-7 compared to the DA-7 group (33 v.s. 40 days, p=0.002). Toxicity was comparable in both groups. The probability of 3-year leukemia-free survival (LFS) for DAC-7 and DA-7 group equaled 43 and 34%, respectively (p=NS). There was a trend toward a higher LFS rate for patients aged >40 years receiving DAC-7 compared with DA-7 regimen (44 v.s. 28%, P=0.05). Recently the same group presented updated results of this study [117]. For patients aged >40 years, the 5-year LFS rate was significantly higher in DAC-7 arm (26%) compared with DA-7 arm (19%) (p=0.02). Moreover, a trend toward higher overall survival rate was observed in patients treated with DAC-7 (23%) compared to DA-7 (16%) regimen (p=0.05). This study proves that addition of 2-CdA increases antileukemic potency of DNR+Ara-C regimen, thus resulting in a higher CR rate after one induction cycle when compared to DA-7 without additional toxicity. It shortens hospitalization time and may improve long-term survival in patients aged >40 years.

Encouraging results with combination regimen of 2-CdA, Ara-C and IDA have been also obtained in elderly patients. Juliusson *et al.* [118] performed a randomized phase II study in previously untreated AML patients over 60 years of age. All patients received Ara-C 1g/m²/day bid for 4 days and IDA 10 mg/m²/day for 2 days. Two thirds were randomized to receive additional 2-CdA 5 mg/m² given before Ara-C bid for 4 days (CCI regimen). The overall CR rate was 62%. There was a significantly higher CR rate after one course CCI (51%) in comparison with the group treated without 2-CdA (35%) (p=0.014). Moreover, 2-CdA did not increase either the rate of early death, or time to recovery from neutropenia and thrombocytopenia, or median time with fever >38°C and intravenous antibiotics treatment. It is also thought that the addition of G-CSF may further improve the effects of 2-CdA in combination with Ara-C. In our preliminary study we evaluated both the efficacy and toxicity of the combination of 2-CdA with high dose Ara-C and G-CSF (CLAG regimen) in 58 patients with refractory or relapsed AML [119,120]. The protocol consisted of an

infusion of 5 mg/m² of 2-CdA over 2 hours daily for 5 consecutive days, a 4-hour infusion of Ara-C (2 g/m²) started 2 hours after each infusion of 2-CdA and G-CSF at a dose of 300 µg s.c., for 6 days, started 24-hours before the first dose of chemotherapy. The rate of CR was 50%. Disease free survival (DFS) after 1 year was 29%. CLAG regimen was more effective in primary resistant patients who did not receive 2-CdA in the first-line induction therapy compared to patients treated previously with 2-CdA. Subsequently, we evaluated the efficacy and toxicity of induction treatment consisting of 2-CdA (5 mg/m²), Ara-C (2 g/m²), MIT (10 mg/m²) and G-CSF (CLAG-M) in 42 refractory AML patients [121]. CR was achieved in 21 (49%) patients. The efficacy of CLAG-M was similar in patients who were treated with and without 2-CdA in the first-line induction therapy. Hematological toxicity was the most prominent toxicity of this regimen. The overall survival (OS) after 1 year for all 42 patients was 43%, whereas OS for 20 patients in CR was 73%. DFS (1 year) was 68.6%. We concluded that CLAG-M is highly active protocol in refractory AML. However, currently there are no randomized studies suggesting a real superiority of a given salvage regimen in AML patients refractory to standard therapy.

Multiple Sclerosis

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) characterized by focal T-cell and macrophage infiltrates that lead to lymphocyte-dependent demyelination process and loss of neurological function [122]. It is typically present with a relapsing and remitting course (RRMS). However, over one-half of patients with RRMS enter a progressive phase defined as secondary progressive MS (SPMS) [123]. Approximately 10% of patients have primary progressive MS (PPMS) characterized by a continuous accumulation of neurological deficits from the beginning of the disease without relapse or remission [124]. In the last decade there is an increasing evidence that damage to the CNS is mediated by autoimmune mechanisms [122, 125]. For this reason immunosuppression is a rational approach to treatment of this disorder. As mentioned above 2-CdA has been found to cause death of lymphocytes by apoptosis and to have relatively low toxicity toward other tissues [8,10]. Its long-lasting lymphocytotoxic activity suggests that 2-CdA could be useful in modulating autoimmune processes involving lymphocyte abnormalities in MS.

Over the decade only a few clinical trials evaluated the influence of 2-CdA on the course of SM (Table 9). Shelby *et al.* [126] investigated the effects of 2-CdA therapy on lymphocyte subsets in 19 patients with severe chronic progressive MS (CPMS). 2-CdA was administered subcutaneously at a daily dose of 0.07 mg/kg for 5 days per cycle, repeated every 4 weeks for a total of 6 cycles. The treatment was well tolerated and no clinically significant side effects were noted. Between baseline and the end of cycle 6 highly significant decreases in the levels of absolute lymphocyte count (ALC), CD19, CD4 ($p<0.0001$) and CD8 counts ($p=0.005$) were observed. The suppression of ALC, CD4 and CD8 subsets was profound and lasted more than one year after completion of therapy. Platelets, granulocytes and red blood cells counts were unaffected.

In the other randomized, placebo-controlled, double-blind study Janiec *et al.* [127] evaluated the influence of immunosuppressive cladribine treatment with total dose of 2.1 mg/kg administered in 7 cycles for 12 months, on serum leukocytes as well as on serum interleukin-2 (IL-2) and soluble IL-2 receptor (sIL-2R) levels assessed before and right after treatment. The study involved 69 patients with CPMS, 34 treated with cladribine and 35 with placebo. In patients treated with cladribine a statistically significant gradual decrease in lymphocyte level was observed from the 7th week ($p=0.05$) to the 12th month ($p<0.0001$) after the treatment initiation. Similarly to the previous study the lymphocyte levels remained decreased compared to the baseline for the next 12 months of follow-up. Moreover, the mean values of IL-2 and sIL-2R serum levels were significantly lower ($p=0.01$ and $p=0.0005$, respectively) after completion of the cladribine treatment as compared to baseline. The similar results were not observed in the placebo group. These observations indicate that 2-CdA treatment may influence immunological process in MS patients.

The efficacy and safety of cladribine therapy in MS patients were first evaluated in a randomized double-blind crossover trial by Sipe *et al.* [128]. The study involved 51 patients (48 as matched pairs) with chronic progressive MS (CPMS) for more than two years. The patients received four monthly courses of 2-CdA administered by continuous 7-day intravenous infusion at a dose of 0.1 mg/kg/day (total dose of cladribine was 2.8 mg per kg) or placebo (saline). The evaluation was based on two neurological assessments, the Scripps Neurologic Rating Scale (SNRS) and the Kurtzke Expanded Disability Status Scale (EDSS), as well as cerebrospinal fluid (CF) and brain magnetic resonance imaging (MRI) examinations. After the first year of the study both neurological scores indicated a modest improvement in patients on 2-CdA and progressive deterioration in patients randomized to placebo ($p<0.004$ for EDSS and $p<0.001$ for SNRS). Moreover, average demyelinated volumes on MRI and concentrations of oligoclonal bands in CF were stable or improved in the patients receiving 2-CdA but continued to deteriorate in patients on placebo. 2-CdA was generally well tolerated and clinically significant toxicity (severe marrow suppression with later complete recovery) occurred only in 1 patient. One patient died of fulminant hepatitis B, which likely was not related to cladribine. Moreover mild episodes of herpes zoster were observed in two patients.

The preliminary findings were confirmed by updated results of this study [129]. During the second year of the study the patients who had received placebo were given active drug but at one-half the total dose (1.4 mg of cladribine per kg) that had been administered during the first year. The patients who had originally received cladribine were crossed over to placebo. In the patients treated with 2.8 mg of cladribine per kg, the improvement in SNRS scores appeared to peak at 18 months and was maintained for 24 months of follow-up. In the patients treated with the lower total dose of cladribine (1.4 mg per kg) during only the second year of the study the stabilization of disease was also observed. However, the time of improvement was shorter with the peak at 8 months after the treatment initiation. Moreover, the treatment with a lower dose of cladribine was

Table 9. Selected clinical trails evaluating the efficacy of 2-CdA in MS

Authors	Study design	Type of MS	Number of patients	Schedule of cladribine therapy	Effectiveness on relapse rate	Effectiveness on disease progression	Outcome MRI	Comments
Sipe <i>et al.</i> [128] Beutler <i>et al.</i> [129]	RCT (double-blind, crossover after 1 year)	CPMS for > 2 years	48	2,8 mg/kg i.v. in year 1, 1,4 mg.kg i.v. in year 2 vs placebo	NA	Improvement of EDSS (F=10,19; p=0,0026) and SNRS (F=23,46; p<0,0001) scores based on 2 years crossover	Stabilization or improvement of average demyelinated volume in cladribine arm	
Rice <i>et al.</i> [130] Filippi <i>et al.</i> [131]	RCT (double blind, placebo controlled)	PPMS, SPMS for >1 year	159	0,7 mg/kg s.c or 2,1 mg/kg s.c vs placebo	NA	No significant difference in EDSS and SNRS between treatment arms Trend toward more favourable clinical response of SPMS patients in cladribine arm compared wit placebo	Significant reduction in the presence, number and volume gadolinium enhanced T1 brain lesions	Very advanced disease stage at baseline (Me EDSS 6.0) Discrepancy between MRI and clinical effect; probably no effect on tissue injury
Romain <i>et al.</i> [132]	RCT (double blind, placebo controlled)	RRMS for >1 year, 2 or more relapses in past 2 years	52	2,1 mg/kg vs placebo	No significant effects of cladribine on relapse rate	No significant differences between treatment groups	Suppression of MRI-enhancing lesions by the 6-month therapy in cladribine arm	Reduction of the combined measure of frequency and severity of relapses in 2-CdA arm
Stelmasiak <i>et al.</i> [133]	Pilot, non-randomized	RRMS	10	5 mg/d s.c x 5 days x 8 courses or 10 mg/d p.o. x 5 days x 8 courses	Reduction of the relapse rate in 7 patients	Improvement in EDSS score (F=18,07; p<0,02)	NA	

Abbreviations: Cladribine - 2-CdA; MS - multiple sclerosis; MRI - magnetic resonance imaging; RCT - randomized clinical trial; CPMS - chronic progressive multiple sclerosis; PPMS - primary progressive multiple sclerosis; SPMS - secondary progressive multiple sclerosis; RRMS - relapsing-remitting multiple sclerosis; i.v. - intravenously; s.c. - subcutaneously; p.o - orally, d-day; NA - not applicable, EDSS - Kurtzke Expanded Disability Status Scale; SNRS - Scripps Neurologic Rating Scale.

also able to significantly reduce the occurrence of enhancing lesions on MRI scans ($p < 0.001$, McNemar test). The toxicity was mild and dose-related.

Recently, Rice *et al.* [130] have evaluated the safety and efficacy of two doses of 2-CdA in 159 patients with progressive MS (30% with PPMS and 70% with SPMS). The patients were randomly assigned to receive either placebo or 2-CdA 0.07 mg/kg/day for 5 consecutive days every four weeks for two (total dose of 0.7 mg/kg) or six (total dose of 2.1 mg/kg) cycles, followed by placebo, for a total of eight cycles. The both 2-CdA regimens were safe and well tolerated. This study showed no significant treatment effect for 2-CdA after one year's observation in terms of changes in EDSS or SNRS scores. However, in patients with SPMS treated with 2-CdA the increase in EDSS score over time (0.0) was less than in placebo group (0.3; $p = NS$). In contrast, in patients with PPMS very little changes in EDSS score were observed in any treatment arms. Similarly, although no significant differences among treatment groups were found

in time to progression for all patients, there was a trend toward a more favorable clinical response to 2-CdA than to placebo in the patients with SPMS. The lack of the overall treatment difference in this study may be in part due to a very advanced disease stage at baseline (median EDSS score for all three arms 6.0). However, in these trials there was a discrepancy between the clinical effects and MRI findings. Both 2-CdA treatment regimens were superior to placebo for the proportion of patients having gadolinium-enhanced T1 lesions and for the mean volume and number of such lesions ($p < 0.003$) on MRI. Moreover, a modest improvement of T2 burden of disease in 2-CdA arms and worsening in placebo group was noted. To better understand this problem Filippi *et al.* [131] compared changes in the whole brain volume measured using MRI scans and evaluated the correlations between the change in the whole brain volume and the change in other conventional MRI measures in the same group of 159 progressive MS patients enrolled to the trial. A significant decrease in the brain volume over time was observed both in the entire population of patients ($p < 0.001$)

and in placebo group ($p < 0.04$). There was no significant effect of 2-CdA therapy on brain volume changes. The authors suggest that MRI-visible inflammation and new lesion formation has a marginal role in the development of brain atrophy in patients with progressive MS. The discrepancy among the effects of 2-CdA on different MRI-derived measures is likely due to the inability of the drug to modify the mechanisms leading to severe tissue destruction in progressive MS.

Evidence on the effectiveness of 2-CdA in the remitting-relapsing MS comes from two smaller trials [132,133]. Romine *et al.* [132] reported the effects of 2-CdA on relapse rate in 52 RRMS patients who entered a double blind, placebo-controlled, randomized trial. The patients received either placebo or 2-CdA 0.07 mg/kg/day by subcutaneous injection for 5 consecutive days as 6 monthly courses for a total cumulative dose of 2.1 mg/kg. Comparison of the combined measure of frequency and severity of relapses using Mantel's extension of the Mantel-Haenzel procedure showed a reduction in the 2-CdA group compared with placebo. However, there was no significant difference in the relapse rate between treatment groups. In the 2-CdA patients MRI-enhancing lesions were completely suppressed by the 6-month therapy. The treatment was well tolerated without any adverse events. Only in two 2-CdA-treated patients mild segmental herpes zoster occurred.

In the second pilot trial 10 patients with RRMS were treated with six courses of 2-CdA at monthly intervals followed by two additional courses at three-month intervals [133]. Each course consisted of 2-CdA given either subcutaneously at a dose of 5 mg/day (6 patients) or orally at a dose of 10 mg per day (4 patients) for 5 consecutive days. These dosing regimens produce equivalent area under the concentration-time curve of the drug. The treatment resulted in the significant improvement of the neurological status (expressed semi quantitatively according to EDSS scale) ($p < 0.02$). The number of relapses compared to the two-year period immediately before the treatment was reduced almost 5 times on average in 7 patients and remained unchanged in 3 patients. Patients who experienced the reduced relapse rate seemed to show longer and more pronounced improvement in their neurological status. The tolerance of therapy was good and hematological side effects were mild. An average decrease in lymphocyte count to 1/3 of the initial value was noted.

In conclusion, the results of the currently available clinical trials indicate that 2-CdA may be effective in the treatment of MS especially in the remitting-relapsed and secondary progression type of disease. The role of 2-CdA in the treatment of more advanced primary progressive MS seems to be less significant. However, further clinical studies should be undertaken to better determine its role.

Other Autoimmune Diseases

Treatment with 2-CdA leads to a decrease in the CD4+/CD8+ ratio for an extensive period of time exceeding even 24 months [134]. Moreover, HCL patients at 3-5 years by the end of treatment with 2-CdA together with a reduction in the absolute number of CD4+ T cells showed a persistent and significant decrease in the proportion and absolute

number of CD4+/CD45 RA+ cells as compared with both untreated HCL patients and normal controls [135]. However, 2-CdA leads also to a significant decrease of CD3+ and CD8+ T lymphocytes and simultaneously a significant increase in the proportion of natural killer (NK) cells and normalization of the increased serum levels of soluble IL-2 [136]. Human monocytes are as sensitive as lymphocytes to 2-CdA. Monocytes exposed *in vitro* to 2-CdA rapidly developed DNA strand breaks. Low 2-CdA concentrations (5-20 μM) inhibited monocyte phagocytosis and higher concentrations led to a dose and time dependent loss of monocyte viability. Moreover, Carrera *et al.* [137] showed that circulating monocytes disappeared within 1 week in patients with rheumatoid arthritis or cutaneous T cell lymphoma during continuous infusion of 2-CdA. These observations have suggested a possible therapeutic role for 2-CdA in the treatment of patients with some refractory autoimmune disorders. However, there is much less experience with this agent in the patients with autoimmune diseases than with hematologic malignancies and apart from MS, well designed prospective trials are lacking.

Some studies have suggested that 2-CdA may be useful in the treatment of autoimmune hemolytic anemia (AIHA) both essential and secondary to CLL [46,138-140]. More recent reports suggest that 2-CdA may induce autoimmune hemolytic anemia (AIHA) in patients with CLL despite the reduction in the leukemic clone [140,141]. However, the results of a randomized study did not support this hypothesis. In our randomized trial, AIHA was noted in seven patients treated with 2-CdA and in two patients treated with chlorambucil, but this difference was not statistically significant ($p = 0.3$) [60]. The probability of drug-induced AIHA seems to be higher if hemolysis occurs shortly (i.e. 1-2 months) after 2-CdA administration [142]. When it occurs later, it is probably not drug related and appears by chance.

Use of 2-CdA in the treatment of refractory factor VIII inhibitors in persons without hemophilia has been recently reported [143]. The drug was administered to 6 patients at a dose of 0.1 mg/kg as a 24-hour continuous infusion for a total of 7 days each cycle. The patients received an average of 3 immunosuppressive regimens prior to enrollment to 2-CdA study. The study demonstrated that 2-CdA is an effective and safe immunosuppressant in patients with inhibitors to factor VIII that is refractory to conventional treatment. The median inhibitor titer against human and porcine factor VIII before treatment with 2-CdA was 31 Bethesda units (BU) and 9 BU, respectively. The median inhibitor titer against human and porcine factor VIII after treatment was 3.5 BU and 1.5 BU, respectively. These results indicate that 2-CdA is an effective agent for inhibitory factor VIII with an acceptable toxicity profile. However, further studies with a larger number of patients and longer follow-up are needed.

Preliminary reports concerning the use of 2-CdA in refractory rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and Sjogren's syndrome have been also published [144-146]. Davis *et al.* [145] investigated safety and tolerability of 2-CdA in patients with SLE associated glomerulonephritis. In a phase I study 12 patients with proliferative lupus nephritis received 2-CdA either in weekly

escalating intravenous injections at a dose of 0.15 mg/kg/week for 4 weeks, 0.1875 mg/kg/week for 4 weeks and 0.225 mg/kg/week for four weeks or in a continuous 7 day infusion at a dose of 0.05 mg/kg/day. Peripheral lymphocyte depletion without a significant myelotoxicity was observed. Naive and memory T cells were decreased as were lymphocytes with markers of early and late activation. Peripheral B cell depletion was not associated with a significant decrease in serum immunoglobulin levels. The authors found that continuous infusion induced better clinical responses than weekly infusions. Three of seven patients treated with continuous 2-CdA infusion responded completely and renal function did not deteriorate in any of the seven patients. Other authors suggest however that 2-CdA may be effective in other manifestations of SLE (cutaneous vasculitis) but it does not seem to have a consistent effect in severe nephritis [146]. Schrimmer *et al.* [144] assessed the safety profile of low dose 2-CdA in patients who received more than three previous lines of treatment. Five patients were treated with a subcutaneous dosage of 0.05 mg/kg 2-CdA weekly over a period of 8 weeks. They found that both T and B cells decreased below the normal range while NK cells were remaining stable. These results indicate that even low dose 2-CdA can decrease T and B lymphocytes in patients with refractory RA. However, possible clinical activity of low-dose 2-CdA in this disease requires further studies.

Anecdotal reports have suggested 2-CdA as a therapeutic agent in patients with psoriasis. Zinzani *et al.* [147] treated with single course of 2-CdA a patient with HCL and psoriasis. They reported the remission of psoriasis lasting 8 months. In other study 2-CdA has been applied for treatment of 6 patients with psoriatic arthritis [148]. Four patients showed improvement of the joint disease and five demonstrated improvement of skin lesion lasting at least 6 months until the follow-up was stopped. Other cases of complete responses of psoriatic skin lesions were also reported [149,150]. These observations suggest a possible therapeutic role of 2-CdA in treatment of patients with advanced and refractory psoriasis. However, further clinical trials in this disease are needed.

CONCLUSIONS

Several clinical trials have shown significant activity of 2-CdA in indolent lymphoid malignancies, especially in HCL, CLL and LG-NHL. Multicenter studies on large groups of patients have demonstrated that one course of 2-CdA therapy induces durable and unmaintained remission in the vast majority of previously untreated and pretreated patients with HCL. At present 2-CdA is the drug of choice in the treatment of this disease. However, in CLL, LG-NHL and AML, in contrast to HCL, 2-CdA seems to be more effective when combined with other antineoplastic agents, probably because of different biology of the underlying disease process. Recent phase II and phase III studies indicate that 2-CdA has similar activity and toxic profile to FA in CLL, LG-NHL and AML. 2-CdA work better in pediatric AML than adult AML but the reason for this difference is unclear. Activity of 2-CdA in autoimmune diseases has been less extensively investigated than in hematological disorders. However preliminary results of

recent clinical trials and anecdotal reports suggest that this agent may be of some value in the treatment of SM, RA, SLE, psoriasis and factor VIII inhibitors. However, further clinical trials evaluating the role of 2-CdA alone and in combination with other immunosuppressive agents in therapy of these diseases are needed.

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REFERENCES

- [1] Beutler E. Cladribine (2-chlorodeoxyadenosine). *Lancet* 1991; 340: 952-6.
- [2] Bryson HM, Sorkin EM. Cladribine. A review of its pharmacodynamic and therapeutic potential in haematological malignancies. *Drugs* 1993; 76: 872-94.
- [3] Carson DA, Wasson DB, Kaye J, *et al.* Deoxycytidine kinase mediated toxicity of deoxyadenosine analogs toward malignant human lymphoblasts *in vitro* and toward murine L1210 leukemia *in vivo*. *Proc Natl Acad Sci USA* 1980; 77: 6865-9
- [4] Kazimierzczuk Z, Cottam HB, Revankar, GR, Robins RK. Synthesis of 2-deoxynucleosides *via* a novel direct stereospecific sodium salt glycosylation procedure. *J Am Chem Soc* 1984; 106: 6379-82.
- [5] Robak T. Cladribine in the treatment of chronic lymphocytic leukemia. *Leuk Lymphoma* 2001; 40: 551-64.
- [6] Pettit AR. Mechanism of action of purine analogues in chronic lymphocytic leukemia. *Br J Haematol* 2003; 121: 692-702.
- [7] Robertson LE, Chubb S, Meyn RE, *et al.* Induction of apoptotic cell death in chronic lymphocytic leukemia 2-chloro-2'-deoxyadenosine and 9- β -D-arabinosyl-2-fluoro-adenine. *Blood* 1993; 81: 143-50.
- [8] Castejon R, Vargas JA, Briz M, *et al.* Induction of apoptosis by 2-chlorodeoxyadenosine in B-cell chronic lymphocytic leukemia. *Leukemia* 1997; 11: 1253-7.
- [9] Genini D, Adachi S, Chao O, *et al.* Deoxyadenosine analogues induce programmed cell death in chronic lymphocytic leukemia cells by damaging the DNA and by directly affecting the mitochondria. *Blood* 2000; 96: 3537-43.
- [10] Smolewski P, Darzynkiewicz Z, Robak T. Caspase-mediated cell death in hematological malignancies: theoretical considerations, methods of assessment, and clinical implications. *Leuk Lymphoma* 2003; 44: 1089-104.
- [11] Johnson JB, Daeninck P, Verburg L, *et al.* p53, mdm-2, bax and bcl-2 and drug resistance in chronic lymphocytic leukemia. *Leuk Lymphoma* 1997; 26: 425-49.
- [12] Saven A, Piro LD. 2-Chlorodeoxyadenosine in the treatment of hairy cell leukemia. *Cancer Invest* 1993; 11: 559-64.
- [13] Juliusson G, Liliemark J. Rapid recovery from cytopenia in hairy cell leukemia after treatment with 2-chloro-2'-deoxyadenosine (CdA): relation to opportunistic infections. *Blood* 1992; 79: 888-94.
- [14] Robak T, Błasińska-Morawiec M, Krykowski E, *et al.* 2-Chlorodeoxyadenosine (2-CdA) in 2-hour versus 24-hour intravenous infusion in the treatment of patients with hairy cell leukemia. *Leuk Lymphoma* 1996; 22: 107-11.
- [15] Liliemark J, Juliusson G. On the pharmacokinetics of 2-chloro-2'-deoxyadenosine in humans. *Cancer Res* 1991; 51: 70-72.
- [16] Robak T, Błasińska-Morawiec M, Krykowski E, *et al.* Intermittent 2-hour intravenous infusions of 2-chlorodeoxyadenosine in the treatment 110 patients with refractory or previously untreated B-cell chronic lymphocytic leukemia. *Leuk Lymphoma* 1996; 22: 509-14.
- [17] Robak T, Błonski JZ, Kasznicki M, *et al.* Cladribine with prednisone versus chlorambucil with prednisone as first line therapy in randomized, multicenter trial. *Blood* 2000; 96: 2723-9.

- [18] Robak T, Błonski JZ, Kasznicki M, *et al.* Cladribine with or without prednisone in the treatment of previously treated and untreated B-cell chronic lymphocytic leukemia: updated results of the multicentre study of 378 patients. *Br J Haematol* 2000; 108: 357-68.
- [19] Robak T, Błonski JZ, Urbanska-Rys H, Błasińska-Morawiec M, Skotnicki AB. 2-chlorodeoxyadenosine (Cladribine) in the treatment of patients with chronic lymphocytic leukemia 55 years old and younger. *Leukemia* 1999; 13: 518-23.
- [20] Robak T, Błasińska-Morawiec M, Błonski JZ, *et al.* The effect of 2-h infusion of 2-chlorodeoxyadenosine (cladribine) with prednisone in previously untreated B-cell chronic lymphocytic leukemia. *Eur J Cancer* 1997; 33: 2347-51.
- [21] Karlsson K, Stromberg M, Liliemark J, *et al.* Oral cladribine for B-cell chronic lymphocytic leukemia: report of a phase II trial with a 3-d, 3-weekly schedule in untreated and pretreated patients and a long-term follow-up of 126 previously untreated patients. *Br J Haematol* 2000; 116: 538-48.
- [22] Betticher DC, Ratschiller D, Hsu Schmitz SF, von Rohr A, Hess U, Zulian G. Reduced dose of subcutaneous cladribine induces identical response rates but decreased toxicity in pretreated chronic lymphocytic leukaemia. Swiss Group for Clinical Cancer Research (SALL). *Ann Oncol* 1998; 9: 721-6.
- [23] Goodman GR, Bethel KJ, Saven A. Hairy cell leukemia: an update. *Curr Opin Hematol* 2003; 10: 258-66
- [24] Chadha P, Rademaker AW, Mendirata P, *et al.* Treatment of hairy cell leukemia with 2-chlorodeoxyadenosine (2-CdA): long-term follow-up of the Northwestern University experience. *Blood* 2005; 106: 241-6.
- [25] Cheson BD, Sorensen JM, Vena DA, *et al.* Treatment of hairy cell leukemia with 2-chlorodeoxyadenosine via the Group Protocol mechanism of the National Cancer Institute: a report of 979 patients. *J Clin Oncol* 1998; 16: 3007-15.
- [26] Juliusson G, Lenkei R, Liliemark J. Flow cytometry of blood and bone marrow cells from patients with hairy cell leukemia: phenotype of hairy cell and lymphocyte subsets after treatment with 2-chlorodeoxyadenosine. *Blood* 1994; 83: 3672-81.
- [27] Robak T, Błasińska-Morawiec M, Krykowski E, *et al.* 2-chlorodeoxyadenosine (2-CdA) in 2-hour versus 24-hour intravenous infusion in the treatment of patients with hairy cell leukemia. *Leuk Lymphoma* 1996; 22: 107-11.
- [28] Piro LD, Carrera CJ, Carson DA, Beutler E. Lasting remissions in hairy cell leukemia induced by a single infusion of 2-chlorodeoxyadenosine. *N Engl J Med* 1990; 322: 1117-21.
- [29] von Rohr A, Schmitz SF, Tichelli A, *et al.* Treatment of hairy cell leukemia with cladribine (2-chlorodeoxyadenosine) by subcutaneous bolus injection: a phase II study. *Ann Oncol* 2002; 13: 1641-9.
- [30] Jehn U, Bartl R, Dietzfelbinger H, Haferlach T, Heinemann V. An update: 12-year follow-up of patients with hairy cell leukemia following treatment with 2-chlorodeoxyadenosine. *Leukemia* 2004; 18: 1476-81.
- [31] Bastie JN, Cazals-Hatem D, Daniel MT, *et al.* Five years follow-up after 2-chlorodeoxyadenosine treatment in thirty patients with hairy cell leukemia: evaluation of minimal residual disease and CD4+ lymphocytopenia after treatment. *Leuk. Lymphoma* 1999; 35: 555-65.
- [32] Zinzani PL, Magagnoli M, Bandandi M, *et al.* Long-term follow-up of hairy cell leukemia patients treated with 2-chlorodeoxyadenosine. *Haematologica* 2000; 85: 922-5.
- [33] Goodman GR, Burian C, Koziol JA, Saven A. Extended follow-up of patients with hairy cell leukemia after treatment with cladribine. *J Clin Oncol* 2003; 21: 891-6.
- [34] Tallman MS, Hakkimian D, Variakojis D, *et al.* A single cycle of 2-chlorodeoxyadenosine results in complete remission in the majority of patients with hairy cell leukemia. *Blood* 1992; 80: 2203-9.
- [35] Hoffman MA, Janson D, Rose E, Rai KR. Treatment of hairy cell leukemia with cladribine: response, toxicity and long-term follow-up. *J Clin Oncol* 1997; 15: 1138-42.
- [36] Filleul B, Delannoy A, Ferrant A, *et al.* A single course of 2-chlorodeoxyadenosine does not eradicate leukemic cells in hairy cell leukemia patients in complete remission. *Leukemia* 1994; 8: 1153-16.
- [37] Robak T, Błasińska-Morawiec M, Błonski J, *et al.* 2-chlorodeoxyadenosine (cladribine) in the treatment of hairy cell leukemia and hairy cell leukemia variant -7-year experience in Poland. *Eur J Haematol* 1999; 62: 49-56.
- [38] Saven A, Burian C, Koziol JA, Piro LD. Long term follow-up of patients with hairy cell leukemia after cladribine treatment. *Blood* 1998; 92: 1918-26.
- [39] Zinzani PR, Tani M, Marchi E, *et al.* Long-term follow-up of front line treatment of hairy cell leukemia with 2-chlorodeoxyadenosine. *Haematologica* 2004; 89: 309-13.
- [40] Lauria F, Bocchia M, Marotta G, Respadori D, Zinzani PL, Rondelli D. Weekly administration of 2-chlorodeoxyadenosine in patients with hairy cell leukemia is effective and reduces infectious complications. *Haematologica* 1999; 84: 22-5.
- [41] Robak T, Kasznicki M, Góra-Tybor J, *et al.* Randomized comparison of weekly administration and daily courses of cladribine in patients with hairy cell leukemia. Updated results. *Blood* 2004; 104 (suppl 1) 948a (abstract 3478).
- [42] Liliemark J, Albertioni F, Hassan M, Juliusson G. On the bioavailability of oral and subcutaneous 2-chloro-2-deoxyadenosine in humans: alternative routes of administration. *J Clin Oncol* 1992; 10: 1514-8.
- [43] Juliusson G, Heldal D, Hippe E, *et al.* Subcutaneous injections of 2-Chlorodeoxyadenosine for symptomatic hairy cell leukemia. *J Clin Oncol* 1995; 13: 989-95.
- [44] Robak T, Kasznicki M. Alkylating agents and nucleoside analogues in the treatment of B-cell chronic lymphocytic leukemia. *Leukemia* 2002; 16: 1015-27.
- [45] Betticher DC, Ratschiller D, Flsu Schmitz SF, *et al.* Reduced doses of subcutaneous cladribine induces identical response rates but decreased toxicity in pretreated chronic lymphocytic leukemia. *Ann Oncol* 1998; 9: 721-6.
- [46] Piro LD, Carrera CJ, Beutler E, Carson DA. 2-Chlorodeoxyadenosine: an effective new agent for the treatment of chronic lymphocytic leukemia. *Blood* 1988; 72: 1069-73.
- [47] Saven A, Carrera C J, Carson D A, Beutler E, Piro L D. 2-chlorodeoxyadenosine treatment of refractory chronic lymphocytic leukemia. *Leuk Lymphoma* 1991; 5(suppl): 133-8.
- [48] Robak T, Błonski JZ, Kasznicki M, *et al.* Cladribine with or without prednisone in the treatment of previously treated and untreated B-cell chronic lymphocytic leukemia: an updated results of the multicenter study of 378 patients. *Br J Haematol* 2000; 108: 357-68.
- [49] Tallman MS, Hakimian D, Zanzig C, *et al.* Cladribine in the treatment of relapsed or refractory chronic lymphocytic leukemia. *J Clin Oncol* 1995; 13: 983-8.
- [50] Juliusson G, Liliemark J. Long term survival following cladribine (2-chlorodeoxyadenosine) therapy in previously treated patients with chronic lymphocytic leukemia. *Ann Oncol* 1996; 7: 373-9.
- [51] Rondelli D, Lauria F, Zinzani PL, *et al.* 2-Chlorodeoxyadenosine in the treatment of relapsed/refractory chronic lymphoproliferative disorders. *Eur J Haematol* 1997; 58: 46-50.
- [52] Robak T, Błasińska-Morawiec M, Krykowski E, *et al.* Intermittent 2-hour intravenous infusion of 2-chlorodeoxyadenosine in the treatment 110 patients with refractory or previously untreated B-cell chronic lymphocytic leukemia. *Leuk Lymphoma* 1996; 22: 509-14.
- [53] Robak T, Błonski J, Kasznicki J, *et al.* The effect of subsequent therapies in patients with chronic lymphocytic leukemia previously treated with prednisone and either 2-CdA or chlorambucil. *Hematologica/Hematology* 2005; 90: 998-1000.
- [54] Robak T, Błonski JZ, Kasznicki M. Does intensive treatment with high dose chlorambucil and prednisone as first line and cladribine as second line influence the survival of the patients with chronic lymphocytic leukemia. *Leuk Lymphoma* 2001; 41: 545-57.
- [55] Robak T. Therapy of chronic lymphocytic leukemia with purine analogs and monoclonal antibodies. *Transf Apher Science* 2005; 32: 33-44.
- [56] 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.
- [57] Juliusson G, Christiansen I, Hansen MM, *et al.* Oral cladribine as primary therapy for patients with B-cell chronic lymphocytic leukemia. *J Clin Oncol* 1996; 14: 2160-6.
- [58] Tallman MS, Wollins E, Jain V, *et al.* Leustatin in the treatment of patients with previously untreated chronic lymphocytic leukemia. *Blood* 1997; 90(suppl 1): 578a (abstract).

- [59] Delannoy A, Martiat P, Gala JL, *et al.* 2-Chlorodeoxyadenosine (CdA) for patients with previously untreated chronic lymphocytic leukemia (CLL) *Leukemia* 1995; 9: 1130-5.
- [60] Robak T, Błonski JZ, Kasznicki M, *et al.* Cladribine with prednisone versus chlorambucil with prednisone as first-line therapy in chronic lymphocytic leukemia: report of a prospective, randomized, multicenter trial *Blood* 2000; 96: 2723-9.
- [61] Cheson BD, Bennet JM, Rai KR, *et al.* Guidelines for clinical protocols for chronic lymphocytic leukemia: recommendation of the National Cancer Institute Sponsored Working Group. *Am J Haematol* 1988; 29: 152-63.
- [62] Karlson K, Stromberg N, Jonsson V. Cladribine (CdA), fludarabine (F) or high-dose intermittent chlorambucil (Chl) as first-line treatment of symptomatic chronic lymphocytic leukemia. First interim analysis of data from the international randomized phase III trial. *Blood* 2004; 104 (suppl 1): 945a (abstract 3470).
- [63] Robak T, Błonski JZ, Góra-Tybor J, *et al.* Cladribine with cyclophosphamide v.s. fludarabine with cyclophosphamide as first line treatment in chronic lymphocytic leukemia an early report of prospective randomized study (PALG CLL3). *Hematologica Hematology J* 2005; 90 (suppl 2): 143-4 (abstract 364).
- [64] Góra-Tybor J, Robak T. Synergistic action of 2-chlorodeoxyadenosine and cyclophosphamide on murine leukemias L1210 and P388. *Acta Haematol Pol* 1993; 24: 177-82.
- [65] Van Den Neste E, Bontemps F, Delacauw A, *et al.* Potentiation of antitumor effect of cyclophosphamide derivatives in B-chronic lymphocytic leukemia cells by 2-chloro-2'-deoxyadenosine. *Leukemia* 1999; 13: 918-25.
- [66] Van Den Neste E, Louviaux I, Michaux JL, *et al.* Phase I/II study of 2-chloro-2'-deoxyadenosine with cyclophosphamide in patients with pretreated B cell chronic lymphocytic leukemia and indolent non-Hodgkin's lymphoma. *Leukemia* 2000; 14: 1136-42.
- [67] Montillo M, Tedeschi A, O'Brien S, *et al.* Phase II study of cladribine and cyclophosphamide in patients with chronic lymphocytic leukemia and prolymphocytic leukemia. *Cancer* 2003; 97: 114-20.
- [68] Robak T, Błonski JZ, Kasznicki M, *et al.* Cladribine combined with cyclophosphamide is highly effective in the treatment of chronic lymphocytic leukemia. *Hematology J* 2002; 3: 244-50.
- [69] Robak T, Góra-Tybor J, Lech-Maranda E, *et al.* Cladribine in combination with mitoxantrone and cyclophosphamide (CMC) in the treatment of heavily pre-treated patients with advanced indolent lymphoid malignancies. *Eur J Haematol* 2001; 66: 188-94.
- [70] Robak T, Błonski JZ, Kasznicki M, *et al.* Cladribine combined with cyclophosphamide and mitoxantrone as front-line therapy in chronic lymphocytic leukemia. *Leukemia* 2001; 15: 1510-6.
- [71] Robak T, Błonski JZ, Góra-Tybor J, *et al.* Cladribine alone or in combination with cyclophosphamide or cyclophosphamide and mitoxantrone as first line treatment in chronic lymphocytic leukemia: an early report of prospective, randomized study. *Blood* 2004, 104: 100a (abstract 337).
- [72] Smolewski P, Szmigielska A, Cebula B, Sobczak A, Darzynkiewicz Z, Robak T. Proapoptotic effect of rituximab alone and in combination with purine nucleoside analogues in B-cell chronic lymphocytic leukemia cells measured by fluorochrome labeled inhibitors of caspase (EIICA) assay. *Blood* 2002; 100 (supl 1): 387a.
- [73] Robak T, Smolewski P, Urbanska-Rys H, Góra-Tybor J, Błonski JZ, Kasznicki M. Rituximab followed by cladribine in the treatment of heavily pretreated patients with indolent lymphoid malignancies. *Leuk Lymphoma* 2004; 45: 937-44.
- [74] Dimopoulos MA, Kantarjian H, Estey E, *et al.* Treatment of Waldenström macroglobulinemia with 2-chlorodeoxyadenosine. *Ann Intern Med*. 1993; 118: 195-8
- [75] Dimopoulos MA, Kantarjian H, Wela D, *et al.* Primary therapy of Waldenström's macroglobulinemia resistant to standard therapy with 2-chlorodeoxyadenosine. *J Clin Oncol* 1994; 12: 2694-8.
- [76] Dimopoulos MA, Weber DN, Delasalle KB, Keating M, Alexanian R. Treatment of Waldenström's macroglobulinemia resistant to standard therapy with 2-chlorodeoxyadenosine: identification of prognostic factors. *Ann Oncol* 1995; 6: 49-52.
- [77] Delannoy A, Van den Neste E, Michaux JL, Bosly A, Ferrant A. Cladribine for Waldenström's macroglobulinemia. *Br J Haematol* 1999; 104: 928-36.
- [78] Betticher DC, Hsu Schmitz SF, Ratschiller D, *et al.* Cladribine (2-CdA) given as subcutaneous bolus injections is active in pre-treated Waldenström's macroglobulinemia. *Br J Haematol* 1997; 99: 358-63.
- [79] Liu ES, Burian C, Miller HE, *et al.* Bolus administration of cladribine in the treatment of Waldenström macroglobulinemia. *Br J Haematol* 1998; 103: 690-5.
- [80] Hellmann A, Lewandowski K, Zaucha JM, Bieniaszewska M, Halaburda K., Robak T. Effect of a 2-hour infusion of 2-chlorodeoxyadenosine in the treatment of refractory or previously untreated Waldenström's macroglobulinemia. *Eur J Haematol* 1999; 63: 35-41.
- [81] Weber DM, Dimopoulos MA, Delasalle K, Rankin K, Gavino M, Alexanian R. 2-Chlorodeoxyadenosine alone and in combination for previously untreated Waldenström's macroglobulinemia. *Semin Oncol* 2003; 30:243-7
- [82] Dimopoulos M, Mertim G, Leblond A., Anagnostopoulos A, Alexanian R. How we treat Waldenström's macroglobulinemia. *Haematologica Hematology J* 2005; 90: 117-25.
- [83] Johnson SA, Oscier DG, Leblond V. Waldenström's macroglobulinemia. *Blood Rev* 2002; 16: 175-84.
- [84] Kay AC, Saven A, Carrera CJ, *et al.* 2-Chlorodeoxyadenosine treatment of low-grade non-Hodgkin's lymphomas. *J Clin Oncol* 1992; 10: 371-7.
- [85] Betticher DC, von Rohr A, Ratschiller D, *et al.* Fewer infections but maintained antitumor activity with lower dose versus standard dose cladribine in pretreated low grade non-Hodgkin's lymphoma. *J Clin Oncol* 1998; 16: 850-8.
- [86] Liliemark J, Porwit A, Juliusson G. Intermittent infusion of cladribine (CdA) in previously treated patients with low-grade non-Hodgkin's lymphoma. *Leuk Lymphoma* 1997; 25: 313-8.
- [87] Robak T, Góra-Tybor J, Krykowski E, *et al.* Activity of 2-chlorodeoxyadenosine (Cladribine) in 2-hour intravenous infusion in 94 previously treated patients with low-grade non-Hodgkin's lymphoma. *Leuk Lymphoma* 1997; 26: 99-105.
- [88] Tulpule A, Schiller G, Harvey-Buchman L, *et al.* Cladribine in the treatment of advanced relapsed or refractory low and intermediate grade non-Hodgkin's lymphoma. *Cancer* 1998; 83: 370-6.
- [89] Ogura M, Morishima Y, Kobayashi Y, *et al.* Durable response but prolonged cytopenia after cladribine treatment in relapsed patients with indolent non-Hodgkin's lymphomas: results of Japanese phase II study. *Int J Haematol* 2004; 80: 267-77.
- [90] Rummel MJ, Chow KU, Jager E, *et al.* Intermittent 2-hour infusion of cladribine as first-line therapy or in first relapse of progressive advanced low-grade and mantle cell lymphomas. *Leuk Lymphoma* 1999; 82: 957-64.
- [91] Kong LR, Huang CF, Hakimian D, *et al.* Long term follow-up and late complications of 2-chlorodeoxyadenosine in previously treated, advanced indolent non-Hodgkin's lymphoma. *Cancer* 1998; 82: 957-64.
- [92] Saven A, Emanuele S, Kosty M, Koziol J, Ellison D, Piro L. 2-chlorodeoxyadenosine activity in patients with untreated indolent non-Hodgkin's lymphoma. *Blood* 1995; 86: 1710-16.
- [93] Fridrik M, Jager G, Kienzer HR, *et al.* Efficacy and toxicity of 2-chlorodeoxyadenosine (Cladribine) – 2h infusion for 5 days –as first-line treatment for advanced low-grade non-Hodgkin's lymphoma. *Eur J Cancer* 1998; 34: 1560-64.
- [94] Liliemark J, Martinsson U, Cavallin-Stahl E, *et al.* Cladribine for untreated or early low-grade non-Hodgkin's lymphoma. *Leuk Lymphoma* 1998; 30: 573-81.
- [95] Laurencet FM, Zulian GB, Guetty-Alberto M, *et al.* Cladribine with cyclophosphamide and prednisone in the management of low-grade lymphoproliferative malignancies. *Br J Cancer* 1999; 79: 1215-9.
- [96] Robak T, Góra-Tybor J, Urbanska-Rys H, *et al.* Combination regimen of 2-chlorodeoxyadenosine (cladribine), mitoxantrone and dexamethasone (CMD) in the treatment of refractory and recurrent low-grade non-Hodgkin's lymphoma. *Leuk Lymphoma* 1999; 32: 359-68.
- [97] van den Neste E, Louviaux I, Michaux JL, *et al.* Phase I/II study of 2-chloro-2'-deoxyadenosine with cyclophosphamide in patients with pretreated B cell chronic lymphocytic leukemia and indolent non-Hodgkin's lymphoma. *Leukemia* 2000; 14: 1136-42.
- [98] Armitage JO, Tobinai K, Hoelzer D and Rummel MJ. Treatment of indolent non-Hodgkin's lymphoma with cladribine as single-agent therapy and in combination with mitoxantrone. *Int J Hematol* 2004; 79: 311-21.

- [99] Riccioni R, Caracciolo F, Galimberti S, Cecconi N, Petrini M. Low dose 2-CdA schedule activity in splenic marginal zone lymphoma. *Hematol Oncol* 2002; 21: 163-8.
- [100] Rummel MJ, Chow KU, Karahas T, *et al.* Reduced -dose cladribine (2-CdA) plus mitoxantrone is effective in the treatment of mantle-cell and low-grade non-Hpdgkin's lymphoma. *Eur J Cancer* 2002; 38: 1739-46.
- [101] Kalinka E, Wajs JJ, Sułek K, *et al.* Randomized multicenter trial of cladribine alone (C) or in combination with cyclophosphamide (CC) and COP in previously untreated low-grade B-cell non-Hodgkin lymphoma patients. The first interim analysis. *Blood* 2004; 104 (suppl 1) 903a (abstract 3305).
- [102] Tondini C, Balzarotti M, Rampinelli I, *et al.* Fludarabine and cladribine in relapsed/refractory low -grade non-Hodgkin's lymphoma: a phase II randomized study. *Ann Oncol* 2000; 11: 231-3.
- [103] Kong LR., Samuelson E, Rosen ST. 2-chlorodeoxyadenosine in cutaneous T-cell lymphoproliferative disorders. *Leuk. Lymphoma* 1997; 26: 89-97.
- [104] Bouwhuis SA, el-Azhary RA, McEvoy MT, *et al.* Treatment of late-stage Sezary syndrome with 2-chlorodeoxyadenosine. *Int. J. Dermatol* 2002; 41: 352-6.
- [105] Tobinai K, Uike N, Saburi Y, Chou T, *et al.* Cladribine/ATL Study Group, Japan. Phase II study of cladribine (2-chlorodeoxyadenosine) in relapsed or refractory adult T-cell leukemia-lymphoma. *Int J Hematol* 2003; 77: 512-7.
- [106] Santana VM, Hurwitz CA, Blakley RL, *et al.* Complete hematologic remissions induced by 2-chlorodeoxyadenosine in children with newly diagnosed acute myeloid leukemia. *Blood* 1994; 84: 1237-42.
- [107] Santana VM, Mirro Jr J, Kearns C, Schell MJ, Crom W, Blakley R.L. Chlorodeoxyadenosine produces a high rate of complete hematologic remission in relapsed acute myeloid leukemia. *J Clin Oncol* 1992; 10: 364-70.
- [108] Krance RA, Hurwitz CA, Head DR, *et al.* Experience with 2-chlorodeoxyadenosine in previously untreated children with newly diagnosed acute myeloid leukemia and myelodysplastic disease. *J Clin Oncol* 2001; 19: 2804-11.
- [109] Vahdat L, Wong E, Wile M, Rosenblum M, Foley KM, Warell Jr RP. Therapeutic and neurotoxic effects of 2-chlorodeoxyadenosine in adults with acute myeloid leukemia. *Blood* 1994; 84: 3429-34.
- [110] Gordon MS, Young ML, Tallman MD, *et al.* Phase II trial of 2-chlorodeoxyadenosine in patients with relapsed/refractory acute myeloid leukemia: a study of the Eastern Cooperative Oncology Group (ECOG), E5995. *Leuk Res* 2000; 24: 871-5.
- [111] Van den Neste E, Martiat P, Mineur P, *et al.* 2-Chlorodeoxyadenosine with or without daunorubicin in relapsed or refractory acute myeloid leukemia. *Ann Hematol.* 1998; 76: 19-23.
- [112] Gandhi V, Estey E, Keating MJ, Chucrallah A, Plunkett W. Chlorodeoxyadenosine and arabinosylcytosine in patients with acute myelogenous leukemia: pharmacokinetic, pharmacodynamic and molecular interactions. *Blood* 1996; 87: 256-64.
- [113] Kornblau SM, Gandhi V, Andreeff HM, *et al.* Clinical and laboratory studies of 2-chlorodeoxyadenosine + cytosine arabinoside for relapsed or refractory acute myelogenous leukemia in adults. *Leukemia* 1996; 10: 1563-9.
- [114] Crews KR, Gandhi V, Srivastava DK, *et al.* Interim comparison of a continuous infusion versus a short daily infusion of cytarabine given in combination with cladribine in pediatric acute myeloid leukemia. *J Clin Oncol* 2002; 20:4217-24.
- [115] Juliusson G, Liliemark J. 2-chlorodeoxyadenosine (CDA) with and without cytosine arabinoside (Ara-C) and idarubicin for acute myeloid and lymphoid leukemia - clinical and pharmacokinetic studies. *Br J Haematol* 1994; 87: 48a (Abstract).
- [116] Hołowiecki J, Grosicki S, Robak T, *et al.* Addition of cladribine to daunorubicin and cytarabine increases complete remission rate after a single course of induction treatment in acute myeloid leukemia. Multicenter phase III study. *Leukemia* 2004; 18: 989-97.
- [117] Hołowiecki J, Grosicki S, Robak T, *et al.* Cladribine added to the standard AML treatment improves long-term outcome in high tumour burden and older than 40 years acute myeloid leukemia patients. Five-year follow-up of the DAC vs. DA study. *Haematologica Hematology J* 2005; 90 suppl 809a (Abstract).
- [118] Juliusson G, Hoglund M, Karlsson K, *et al.* Increased remissions from one course for intermediate-dose cytosine arabinoside and idarubicin in elderly acute myeloid leukemia when combined with cladribine. A randomized population based phase II study. *Br J Haematol* 2003; 123: 810-8.
- [119] Robak T, Wrzesien-Kus A, Lech-Maranda E, Kowal M, Dmoszynska A. Combination regimen of cladribine (2-chlorodeoxyadenosine), cytarabine and G-CSF (CLAG) as induction therapy for patients with relapsed or refractory acute myeloid leukemia. *Leuk Lymphoma* 2000; 39: 121-9.
- [120] Wrzesien-Kus A, Robak T, Lech-Maranda E, *et al.* Polish Adult Leukemia Group. A multicenter, open, non-comparative, phase II study of the combination of cladribine (2-chlorodeoxyadenosine), cytarabine and G-CSF as induction therapy in refractory acute myeloid leukemia - a report of the Polish Adult Leukemia Group (PALG) *Eur J Haematol* 2003; 71: 155-62.
- [121] Wrzesien-Kus A, Robak T, Wierzbowska A, *et al.* A multicenter open, noncomparative, phase II study of the combination of cladribine (2-chlorodeoxyadenosine), cytarabine, granulocyte colony-stimulating factor and mitoxantrone as induction therapy in refractory acute myeloid leukemia: a report of the Polish Adult Leukemia Group. *Ann Hematol* 2005; 84: 557-64.
- [122] Hafler DA, Weiner MS: a CNS and systemic autoimmune disease. *Immunol Today*, 1989; 10: 104-7.
- [123] Lublin FD, Reingold SC. Defining the clinical course of multiple sclerosis: results of the international survey. *Neurology* 1969; 46: 907-11.
- [124] Thompson AJ, Polman CH, Miller DH, *et al.* Primary progressive multiple sclerosis. *Brain* 1997; 120: 1085-96.
- [125] Hartung HP: Pathogenesis od inflammatory demyelination: implications for therapy. *Curr Opin Neurol*, 1995; 8: 191-9.
- [126] Selby R, Brandwein J, O'Connor P. Safety and tolerability of subcutaneous cladribine therapy in progressive multiple sclerosis. *Can J Neurol Sci* 1998; 25:295-9.
- [127] Janiec K, Wajgt A, Kondera-Anasz Z. Effect of immunosuppressive cladribine treatment on serum leukocytes system in two-year clinical trial in patients with chronic progressive multiple sclerosis. *Med Sci Monitor* 2001; 7: 93-8.
- [128] Sipe JC, Romine JS, Koziol JA, *et al.* Cladribine in treatment of chronic progressive multiple sclerosis. *Lancet* 1994; 344: 9-13.
- [129] Beutler E, Sipe JC, Romine JS, *et al.* The treatment of chronic progressive multiple sclerosis with cladribine. *Proc Natl Acad Sci USA* 1996; 93: 1716-20.
- [130] Rice GPA, Filippi M, Comi G. Cladribine and progressive MS. Clinical and MRI outcomes of multicenter controlled trial. *Neurology* 2000; 54:1145-55.
- [131] Filippi M, Rovaris M, Iannucci G, *et al.* Whole brain volume changes in patients with progressive MS treated with cladribine. *Neurology* 2000; 55: 1714-8.
- [132] Romine JS, Sipe JC, Koziol JA, *et al.* A double-blind, placebo-controlled, randomised trial of cladribine in relapsing-remitting multiple sclerosis. *Proc Assoc Am Physicians* 1999; 111; 35-44.
- [133] Stelmasiak Z, Solski J, Nowicki J, *et al.* A pilot study of cladribine (2-chlorodeoxy-adenosine) in remitting-relapsing multiple sclerosis. *Med Sci Monitor* 1998; 4: 4-8.
- [134] Seymour JF, Kurzrock R, Freireich EJ, Estey EH. 2-chlorodeoxyadenosine induces durable remissions and prolonged suppression of CD4+ lymphocyte counts in patients with hairy cell leukemia. *Blood* 1994; 83: 2906-11.
- [135] Raspadori D, Rondelli D, Birtolo S, *et al.* Long-lasting decrease of CD4+/CD45 RA+ T cells in HCL patients after 2-chlorodeoxyadenosine (2-CdA) treatment. *Leukemia* 1999; 13: 1254-7.
- [136] Lauria F, Rondelli D, Raspadori D, Benfenati D, Tura S. Rapid restoration of natural killer activity following treatment with 2-chlorodeoxyadenosine in 22 patients with hairy cell leukemia. *Eur J Haematol* 1994; 52: 16-20.
- [137] Carrera CJ, Terai C, Lotz M, *et al.* Potent toxicity of 2-chlorodeoxyadenosine toward human monocytes *in vitro* and *in vivo*. A novel approach to immunosuppressive therapy. *J Clin Invest* 1990; 86: 1480-8.
- [138] Carson DA, Piro LD, Wasson DB, Carrera CJ, Beutler E. Activity of 2-chloro-2'-deoxyadenosine in chronic lymphocytic leukemia, hairy cell leukemia and autoimmune hemolytic anemia. *Adv Exp Med Biol* 1989; 253A: 427-31.
- [139] Zaucha JM, Halaburda K, Ciepluch H, Hellmann A. 2-chlorodeoxyadenosine treatment of patients with chronic lymphocytic leukemia associated with autoimmune haemolysis. *Acta Haematol Pol* 1994; 25: 119-27.

- [140] Robak T, Błasińska-Morawiec M, Krykowski E, Hallmann A, Konopka L. Autoimmune haemolytic anemia in patients with chronic lymphocytic leukemia treated with 2-chlorodeoxyadenosine (2-CdA). *Eur J Haematol* 1997; 58: 109-13.
- [141] Chasty RC, Myint H, Oscier DG, *et al.* Autoimmune haemolysis in patients with B-CLL treated with chlorodeoxyadenosine (CDA). *Leuk Lymphoma* 1998; 29: 391-98.
- [142] Fleischman RA, Croy D. Acute onset of severe autoimmune hemolytic anemia after treatment with 2-chlorodeoxyadenosine for chronic lymphocytic leukemia. *Am J Hematol* 1995; 48: 293.
- [143] Sallah S, Wan JY. Efficacy of 2-chlorodeoxyadenosine in refractory factor VIII inhibitors in persons without hemophilia. *Blood* 2003; 101: 943-5.
- [144] Schirmer M, Mur E, Pfeiffer KP, Thaler J, Konwalinka G. The safety profile of low-dose cladribine in refractory rheumatoid arthritis. A pilot trial. *Scand J Rheumatol* 1997; 26: 376-9.
- [145] Davis JC Jr, Austin H 3rd, Boumpas D, *et al.* A pilot study of 2-chloro-2'-deoxyadenosine in the treatment of systemic lupus erythematosus – associated glomerulonephritis. *Arthritis Rheum* 1998; 41: 35-43.
- [146] Kontogiannis V, Lanyon PC, Powell RJ. Cladribine in the treatment of systemic lupus erythematosus nephritis. *Ann Rheum Dis* 1999; 58: 653.
- [147] Zinzani PL, Ricci P, Bendandi M, Tura S. 2-Chlorodeoxyadenosine in psoriasis treatment. *Ann Oncol* 1995; 6: 509-510.
- [148] Eibschitz B, Baird SM, Weisman MH, *et al.* Oral 2-chlorodeoxyadenosine in psoriatic arthritis. *Arthritis Rheum* 1995; 38: 1604-1609.
- [149] Ilyas W, Myers D, Mann R, Seraly MP. Remission of psoriasis after treatment with interferon-alpha and 2 chlorodeoxyadenosine for hairy cell leukemia. *J Am Acad Dermatol* 1999; 40: 316-8.
- [150] Valencak J, Trantinger F, Fiebiger WCC, Raderer M. Complete remission of chronic plaque psoriasis and gastric marginal zone B-cell lymphoma of MALT type after treatment with 2-chlorodeoxyadenosine. *Ann Hematol* 2002; 81: 662-5.

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